CANADIAN THESES ON MICROFICHE

I.S.B.N.

THESES CANADIENNES SUR MICROFICHE

National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL-339 (r. 82/08)

Canada

National Library of Canada
Canadian Theses Division
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Division des thèses canadiennes

54037

0-315-06117-0

## PERMISSION TO MICROFILM — AUTORISATION DE MICROFILMER

- Please print or type — Écrire en lettres moulées ou dactylographier

Full Name of Author — Nom complet de l'auteur

Peter Richard Smith

| | |
|---|---|
| Date of Birth — Date de naissance<br>May 7, 1957 | Country of Birth — Lieu de naissance<br>Canada |

Permanent Address — Résidence fixe

9211 - 118 St.
Edmonton, Alberta
T6G 1T8 Canada

Title of Thesis — Titre de la thèse

Larval Development and Metamorphosis of *Sabellaria cementarium* Moore (Polychaeta: Sabellariidae)

University — Université

University of Alberta

Degree for which thesis was presented — Grade pour lequel cette thèse fut présentée

Master of Science

| | |
|---|---|
| Year this degree conferred — Année d'obtention de ce grade<br>1981 | Name of Supervisor — Nom du directeur de thèse<br>Dr. F.S. Chia |

- Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to microfilm this thesis and to lend or sell copies of the film.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de microfilmer cette thèse et de prêter ou de vendre des exemplaires du film.

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

| | |
|---|---|
| Sept. 30, 1981.<br>Date | Peter R. Smith<br>Signature |

NL-91 (4/77)

THE UNIVERSITY OF ALBERTA

# Larval Development and Metamorphosis of *Sabellaria cementarium* Moore (Polychaeta: Sabellariidae)

by

© Peter Richard Smith

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF ZOOLOGY

EDMONTON, ALBERTA
FALL, 1981

THE UNIVERSITY OF ALBERTA

## RELEASE FORM

NAME OF AUTHOR ........ Peter Richard Smith

TITLE OF THESIS ........ Larval Development and Metamorphosis of *Sabellaria cementarium* Moore (Polychaeta: Sabellariidae)

DEGREE FOR WHICH THESIS WAS PRESENTED ........ Master of Science

YEAR THIS DEGREE GRANTED ........ 1981

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(signed) Peter R. Smith

PERMANENT ADDRESS:

9211 - 118 Street
Edmonton, Alberta
Canada, T6G 1T8

DATED August, 7 1981

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Larval Development and Metamorphosis of *Sabellaria cementarium* Moore (Polychaeta: Sabellariidae)" submitted by Peter Richard Smith in partial fulfilment of the requirements for the degree of Master of Science.

F. S. Chia
Supervisor

B. S. Heming

Date August 7, 1981

## ABSTRACT

A laboratory study of the larval development and metamorphosis of the polychaete *Sabellaria cementarium* was carried out. At 10-14°C development proceeds as follows: 23 hours, early trochophore with prototroch and apical tuft; 65 hours, 1 pair of provisional setae; 3.5 days, feeding trochophore; 18 days, metatrochophore; 4 weeks, metatrochophore with tentacle buds; 5-6 weeks, nectochaeta competent to metamorphose; 6-8 weeks, settlement and metamorphosis. Defensive, feeding and settling behaviors are described.

Tube sand of adult *S. cementarium*, *Phragmatopoma lapidosa*, and *Idanthrysus ornamentatus* and beach sand induced settlement and metamorphosis. The larvae exhibit a low degree of substrate specificity in their settlement but sand is essential.

Larval development was arbitrarily divided into 10 stages and histological examination was made of each of these to study the organogenesis and histogenesis of the planktotrophic larvae. The structures examined were: body wall, muscle system, coelom, alimentary tract, nervous system, circulatory system, gland cells and larval tentacles.

Metamorphosis involves a loss of provisional setae, anterior rotation of tentacles and opercular cirri, and reduction of the episphere. Following these changes, the juvenile builds a mucoid tube to which sand grains are cemented. Histological changes during metamorphosis were examined in one, two, and three day post-settled juveniles. The structures examined were: body wall, muscle system, coelom, alimentary tract, nervous system and gland cells. The major histological changes are atrophy of the prototrochal cells, discharge of epispheral and pygidial mucoid glands, discharge of parathoracic glands which form the mucoid tube, formation of head coelom, enlargement of cerebral ganglion, formation of optic ganglia, histolysis of setal sac-esophageal muscle complex, and hypertrophy and dissociation of stomach cells.

Metamorphosis is considered complete when the caudal appendage is formed, 7-10 days post-settlement. By 38 days post-settlement, the juvenile has 3 pairs of tentacles, larval pigmentation has been lost and a second parathoracic segment has formed. Within the opercular crown, the settling paleae have been replaced by the primary opercular paleae.

# ACKNOWLEDGMENTS

My sincere thanks to Dr. A.O.D. Willows, Director, Dr. E.N. Kozloff, Acting Director, and Dr. R.R. Strathmann, Assistant Director, who provided research facilities at Friday Harbor Laboratories, University of Washington. Thanks also to Dr. D.K. Malhotra and Mr. G.D. Braybrook for allowing me use of the transmission and scanning electron microscopes, respectively; Dr. K.J. Eckelbarger (Harbor Branch Foundation) who provided advice on fixation techniques and to Dr. V.J. Martin (University of Louisville) who reviewed portions of this thesis. Special thanks to my friend, Mr. Ron Koss, who introduced me to the microscopical and histological techniques used in carrying out this research. I also wish to thank the members of my examination committee, Drs. F.S. Chia, J.R. Nursall, D.M. Ross, H.F. Clifford and B. Heming for critically reviewing this thesis.

I am extremely grateful to my supervisor, Professor Fu-Shiang Chia, who has been unfailing in his encouragement and advice during my stay at the University of Alberta.

Finally, I wish to thank my parents for their support and encouragement during my undergraduate and graduate years.

This research was supported by a Natural Sciences and Engineering Research Council of Canada grant to Professor F.S. Chia.

## TABLE OF CONTENTS

INTRODUCTION .......................................... 1

MATERIALS AND METHODS .............................. 6

RESULTS ................................................ 13

A. Larval Development and Behavior .............. 13

- 18 Hour Pretrochophore ..................... 15
- 23 Hour Trochophore ......................... 15
- 44-46 Hour Trochophore ..................... 16
- 60-70 Hour Trochophore ..................... 16
- 3.5-4 Day Trochophore ...................... 17
- 5 Day Trochophore ........................... 18
- 12 Day Trochophore .......................... 19
- Metatrochophore ............................. 19
- Metatrochophore with Tentacle Buds ........ 21
- Nectochaeta (Competent Larva) ............. 22
- Provisional Setae and Defensive Behavior ... 27
- Feeding Behavior ............................ 28

B. Organogenesis and Histogenesis of the Planktonic Larvae ........................ 30

- Body Wall ................................... 31
  - i) Cuticle ................................. 31
  - ii) Epidermis ............................. 31
  - iii) Setal Sacs ............................ 32
- Muscle System ............................... 34
- Coelom ...................................... 37
- Alimentary Tract ............................ 38

Nervous System .............................. 45

Circulatory System .......................... 47

Gland Cells ................................. 50

Tentacles ................................... 54

C. Settlement and Metamorphosis ................. 57

Induction of Metamorphosis ................ 57

Morphology and Behavior of the
Metamorphosing Larvae ..................... 58

Histological Changes During
Metamorphosis ............................. 61

Body Wall ............................... 62

i) Cuticle ............................. 62

ii) Epidermis .......................... 63

iii) Setal Sacs ........................ 64

Muscle System ........................... 65

Coelom .................................. 66

Alimentary Tract ........................ 67

Nervous System .......................... 69

Gland Cells ............................. 70

D. Development of Juvenile Worms ................ 73

5 Days Post-settlement ..................... 73

18 Days Post-settlement .................... 74

38 Days Post-settlement .................... 76

DISCUSSION ...................................... 78

A. Larval Development and Behavior .............. 78

B. Organogenesis and Histogenesis of the
Planktonic Larvae ......................... 86

Body Wall .................................. 87
i) Cuticle ................................. 87
ii) Epidermis .............................. 90
iii) Setal Sacs ............................ 91
Muscle System .............................. 93
Coelom and Segmentation .................... 94
Alimentary Tract ........................... 96
Nervous System ............................. 99
Circulatory System ......................... 101
Gland Cells ................................ 103
Tentacles .................................. 106
C. Settlement and Metamorphosis ............ 109
Induction of Metamorphosis ................. 111
Morphology and Behavior of the Metamorphosing Larvae ............... 114
Histological Changes During Metamorphosis ........................ 116
Body Wall .................................. 116
i) Cuticle ................................. 116
ii) Epidermis .............................. 117
iii) Setal Sacs ............................ 118
Muscle System .............................. 119
Coelom and Segmentation .................... 120
Alimentary Tract ........................... 121
Nervous System ............................. 124
Gland Cells ................................ 126
D. Development of Juvenile Worms ........... 128

TABLES AND FIGURES .................................. 130
LITERATURE CITED .................................... 249

LIST OF TABLES

| TABLE | DESCRIPTION | PAGE |
|---|---|---|
| 1 | Chronology of Larval Development and Metamorphosis of _Sabellaria cementarium_ in the Laboratory | 131 |
| 2 | Summary of the Gland Cell Types in the Parathoracic Region of the Competent Larva | 133 |
| 3 | Induction of Metamorphosis in _Sabellaria cementarium_ | 134 |
| 4 | Summary of the Morphological Changes at Metamorphosis in _Sabellaria cementarium_ | 135 |

# LIST OF FIGURES

FIGURE DESCRIPTION PAGE

1 Diagram of the anterior end of *Sabellaria* ... 137

2 Scanning electron micrographs (SEM) of the anterior end of adult *Sabellaria cementarium* .................................. 139

3 Early embryogenesis of *S. cementarium* ....... 141

4 Development of the planktonic larvae of *S. cementarium*, I ............................ 143

5 Development of the planktonic larvae of *S. cementarium*, II ........................... 145

6 Development of the planktonic larvae of *S. cementarium*, III .......................... 147

7 SEM of 23 hour trochophore and 65 hour trochophore ................................. 149

8 SEM of 5 day old trochophore ................ 151

9 SEM of metatrochophore larva and larva with tentacle buds .............................. 153

10 SEM of competent larvae ..................... 155

11 SEM of a competent larva showing the tentacle and mouth ........................... 157

12 SEM of a competent larva showing the uncini and setae .............................. 159

13 SEM of a competent larva showing the epidermal gland pores ................................ 161

14 SEM of episphere and pygidium of a competent larva ............................. 163

15 Diagrammatical representation of the behavior of the competent and metamorphosing larvae of *S. cementarium* ..... 165

16 Defensive behavior of the planktonic larva .. 167

17 Transmission electron micrographs (TEM) of the cuticle of a 5 day trochophore ....... 169

18 TEM of prototrochal cells and esophagus of a 5 day trochophore ...................... 171

| FIGURE | DESCRIPTION | PAGE |
|---|---|---|
| 19 | Setal sacs | 173 |
| 20 | TEM of an oblique section through the setal sac of a 5 day trochophore | 175 |
| 21 | TEM of sections through the provisional setae of a 5 day trochophore | 177 |
| 22 | Development of muscle system, I | 179 |
| 23 | Development of muscle system, II | 181 |
| 24 | Development of muscle system, III | 183 |
| 25 | Development of coelomic cavities | 185 |
| 26 | Development of alimentary tract, I | 187 |
| 27 | Development of alimentary tract, II | 189 |
| 28 | Development of alimentary tract, III | 191 |
| 29 | Development of alimentary tract, IV | 193 |
| 30 | TEM of the stomach of a 5 day trochophore | 195 |
| 31 | Development of nervous system, I | 197 |
| 32 | Development of nervous system, II | 199 |
| 33 | Development of nervous system, III | 201 |
| 34 | Circulatory system | 203 |
| 35 | Diagrammatical representation of blood vessel formation | 205 |
| 36 | Development of gland cells, I | 207 |
| 37 | Development of gland cells, II | 209 |
| 38 | Development of gland cells, III | 211 |
| 39 | TEM of apical gland cells in a 5 day trochophore | 213 |
| 40 | Larval tentacles | 215 |

| FIGURE | DESCRIPTION | PAGE |
|---|---|---|
| 41 | Diagrammatical representation of gregarious settlement of the larvae of S. cementarium in the dish containing the tube sand of Idanthrysus ornamentatus .................... | 217 |
| 42 | Diagrammatical representation of gregarious settlement of the larvae of S. cementarium in the dish containing the tube sand of Phragmatopoma lapidosa ...................... | 219 |
| 43 | Metamorphic changes in the cuticle and the coelomic cavities ...................... | 221 |
| 44 | Metamorphic changes in the epidermis ........ | 223 |
| 45 | Metamorphic changes in the setal sacs ....... | 225 |
| 46 | Metamorphic changes in the muscle system, I . | 227 |
| 47 | Metamorphic changes in the muscle system, II. | 229 |
| 48 | Metamorphic changes in the alimentary tract, I ...................................... | 231 |
| 49 | Metamorphic changes in the alimentary tract, II ..................................... | 233 |
| 50 | Metamorphic changes in the nervous system ... | 235 |
| 51 | Metamorphic changes in the gland cells ...... | 237 |
| 52 | Micrographs of the adult ..................... | 239 |
| 53 | Juvenile stages of S. cementarium ........... | 241 |
| 54 | SEM of juvenile worm 18 days after settlement ................................. | 243 |
| 55 | SEM of a juvenile 18 days after settlement showing the abdomen and operculum ................................ | 245 |
| 56 | Juvenile S. cementarium, 38 days post-settlement ........................... | 247 |

# INTRODUCTION

The Sabellariidae is a relatively small family of sedentary polychaetes consisting of only 7 genera and 61 species (Fauchald, 1977). The sabellariids are reef builders and most are found on hard substrates from the intertidal to slope depths. The characteristics which describe the family are given by Fauchald (1977) as follows:

> "Tubiculous polychaetes with the body in three regions; posterior region an asetigerous anal tube. Prostomium a narrow ridge fused laterally to the first setiger. Setae of the first setiger forming an operculum with setae in one to three rows. Thorax with two rudimentary segments and three or four parathoracic setigers. Median region with notopodial pectinate uncini and ventral capillary setae".

The characteristics of the anterior region of the sabellariids are shown in Figures 1 and 2.

There have been numerous published accounts of early development in the family Sabellariidae (Horst, 1881; Drasche, 1885; Novikoff, 1937, 1938; Caullery, 1914; Winesdorfer, 1967). The complete larval development through settlement and metamorphosis has been described in three species from Europe: Sabellaria alveolata (Wilson, 1929, 1968a,b, 1970a; Cazaux, 1964), S. spinulosa (Wilson, 1929, 1970b), and Lygdamis muratus (Wilson, 1977; Bhaud, 1969, 1975); three species from the Atlantic coast of North America: Sabellaria vulgaris (Curtis, 1973, 1978; Eckelbarger, 1975), S. floridensis (Eckelbarger, 1977) and Phragmatopoma lapidosa (Eckelbarger, 1976; Eckelbarger and Chia, 1976); two species from China: S. ishikawi (Wu and Ruiping, 1979) and L. giardi (Wu and Ruiping, 1979); a single species from California: P. californica (Dales, 1952; Eckelbarger, 1977); and a single species from the Indian ocean: L. indicus (Bhaud, 1975).

Sabellariids have, in general, indirect development with pelagic, planktotrophic larvae. The duration of planktonic life may range from 14-30 days as in P. lapidosa (Eckelbarger, 1976) to over 32 weeks as in S. alveolata (Wilson, 1970a). The larvae of sabellariids are seasonally abundant in the nearshore plankton in many parts of the world (Bhaud, 1972; Curtis, 1973, 1978; Eckelbarger, 1976; Wilson, 1929) and they are also found among the teleplanic larvae of the open ocean (Scheltema, 1971).

Sabellariid larvae have played an important role in the demonstration that marine invertebrate larvae exhibit substrate selectivity in their settlement. The studies by Wilson (1968a,b, 1970a,b) have shown that sabellariid larvae are induced to settle by the tube cement of their own species. The larvae must make contact with the inducing factor in the cement, an insoluble compound, before the metamorphic response is elicited. Eckelbarger (1978) has recently published a review of settlement and metamorphosis in the Sabellariidae.

Although the larval development and metamorphosis have received considerable attention, only the studies of Eckelbarger (1978) and Eckelbarger and Chia (1978) on

P. lapidosa provide histological information. Eckelbarger (1978) gives fine structural details of certain features of the competent larva, primarily the ciliated and gland cells and Eckelbarger and Chia (1978) provide a fine structural study of the morphogenesis of the larval cuticle. Therefore, we have no knowledge concerning the histogenesis of the planktotrophic larvae and the histological changes at metamorphosis.

Within the Polychaeta, there have been numerous studies dealing with the external features of larval development and metamorphosis (reviewed by Schroeder and Hermans, 1975) which have provided valuable information on the life-histories of polychaetes and the diversity of the developmental patterns and larval types.

The studies of larval histogenesis are those of Åkesson (1961, 1962, 1967a) on Pisione remota, a pisionid with planktotrophic larvae, Tomopteris helgolandica, a tomopterid with pelagic, lecithotrophic larvae, and Eunice kobiensis, a eunicid with benthic larvae; Anderson (1959) on Scoloplos armiger, an orbinid with direct development; Korn (1958) on Harmothoë imbricata, a polynoid with planktotrophic larvae which are brooded until the late trochophore; Potswald (1965, 1978) on Spirorbis mörchi, a spirorbid with brooded lecithotrophic larvae; Segrove (1941) on Pomatoceros triqueter, a serpulid with planktotrophic larvae; Wilson (1932, 1936a) on Owenia fusiformis, an owenid with planktotrophic, mitraria larvae and

Branchiomma vesiculosum, a sabellid with pelagic, lecithotrophic larvae; and Woltreck (1905) on the endo-larva of Polygordius, an archiannelid. The histological changes at metamorphosis have been studied in P. remota (Åkesson, 1961), S. mörchi (Potswald, 1965, 1978), P. triqueter (Segrove, 1941), B. vesiculosum (Wilson, 1936a), O. fusiformis (Wilson, 1932), Polygordius (Woltreck, 1905), and Arenicola cristata (Marsden and Pawson, 1981). Considering there are over 5,300 species of polychaetes, our knowledge concerning the histogenesis, organogenesis, and histological changes at metamorphosis is very limited, particularly in polychaetes with planktotrophic larvae.

Sabellaria cementarium was first described by Moore (1906) from Port Townsend, Washington. In conformance with the generic characteristics of Sabellaria, S. cementarium has the operculum composed of three rows of paleae, the opercular peduncles are short and fused, and the middle opercular paleae are pointed distad (Fauchald, 1977). S. cementarium is distinguished from other members of the genus by the outer opercular paleae which terminate in a flat blade with a distal spinose arista (Fig. 2A,B).

The distribution of S. cementarium is from Alaska to Southern California and east to Japan (Hartman, 1944, 1969). It is found both intertidally and subtidally to shelf depths on rocky bottoms and may be solitary or colonial, often forming reefs (Hartman, 1944, 1969; Smith and Carlton, 1975). In the waters of British Columbia and Washington it is found subtidally at depths of 16-40 fathoms. The tubes occur in small aggregations on rocks and scallop shells of the genus *Chlamys*. They often occur sympatrically within the same aggregations with the only other sabellariid of this region, *Idanthrysus ornamentatus* (Kozloff, 1974). The aggregations of tubes occupy primary space within the benthic community and provide a substrate for epifaunal organisms such as ectoprocts, spirorbids, and serpulids. The hermit crab *Discorsopagurus schmitti* is found occupying the empty tubes of *S. cementarium* (Caine, 1980) as is the polynoid polychaete, *Lepidonotus squmatus*.

The only previous investigations of this species were that of Winesdorfer (1967) and Strathmann (1974), who described briefly the early larval development.

The present study of *Sabellaria cementarium* was undertaken to describe the larval development and metamorphosis and provide detailed information on the histogenesis, organogenesis, and the histological changes at metamorphosis in a typical planktotrophic polychaete larva. Moreover, the results will be compared with what is known for other polychaete larvae.

# MATERIALS AND METHODS

The larval development of Sabellaria cementarium was studied in the summer of 1979 and again in the spring and summer of 1980 at Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington. Adults were collected by dredging at depths of 30-32 fathoms in Griffin Bay, San Juan Island, Washington. Adults were maintained in the laboratory in a sea table with running sea water at 10-14°C. Adults were able to obtain suspended food particles directly from the sea water system and could be kept indefinitely.

Gametes were obtained following the methods of Winesdorfer (1967), Strathmann (1974), and Eckelbarger (1975). Upon removal from their tubes, males and females were placed in individual Pyrex custard cups containing 20 ml of Millepore prefiltered sea water where they spawned. The eggs were collected and placed in Pyrex custard cups with 100 ml of prefiltered sea water. The methods of sperm activation suggested by Winesdorfer (1967) and Strathmann (1974) were not attempted. Active sperm was obtained directly from the males and several drops of sperm suspension were added to the eggs. To avoid polyspermy, the eggs were hand centrifuged and resuspended in prefiltered sea water following the addition of the sperm. The resulting cultures each contained eggs from several females which had been fertilized by the sperm from more than one male. Dishes containing the fertilized eggs were maintained in the sea table at 10-14°C.

At the first appearance of free swimming larvae, they were transferred into 1000 ml beakers containing prefiltered sea water. Two species of algae were added to the cultures as food. They were Dunaliella sp., a green alga and Isochrysis sp., a golden brown alga (both were obtained from Carolina Biological Supply Co.). These algae were cultured in Alga-Gro concentrate media (Carolina Biological Supply Co.) under continuous illumination. Prior to the addition of the algae to the larval cultures, the algae were hand centrifuged to remove the culture media. They were then resuspended in prefiltered sea water. The algal cell concentration was determined using a haemocytometer and added to the larval cultures in a 3:1 mixture of Dunaliella and Isochrysis (Eckelbarger, 1975) to give a final concentration of $10^4$ algal cells per ml. Larval cultures were maintained at 10-14°C in a sea table.

Eckelbarger (1975) has shown that in larval cultures aerated by air stones the larvae will grow faster and settle earlier. Aeration of the cultures was attempted in this study, however, it was discontinued because aeration caused an increase in larval mortality.

Water in the culture dishes was changed every two to three days. This was accomplished by siphoning the culture water from the beakers through a Nitex mesh sieve.

The sieve was made from a small plastic funnel to which Nitex mesh of 53 µm pore size was glued to the mouth and a piece of Tygon tubing was glued to the spout through which the water was siphoned (L. McEdward, personal communication). The larvae were poured into a custard cup which was placed under a Wild M-5 dissecting microscope and the larvae were individually pipetted into a beaker of prefiltered sea water to which algae had been added.

Frequent observations were made to determine the chronology of early development from the time of fertilization. Once the larvae reached the late trochophore stage, observations were made daily. For observations and photographs, the larvae were placed in a drop of water under a coverslip supported by clay feet on the four corners. The water was slowly removed using Bibulous paper so that the larvae were trapped between the slide and the coverslip. It was necessary to relax the later stage larvae using isotonic (7.5%) magnesium chloride. Photographs of the larvae were taken on a Zeiss compound microscope equipped with Nomarski differential interference contrast microscopy and bright field microscopy. Photographs were taken on either Kodak Pan-X or Kodak Plus-X film. Larvae were also photographed using Kodachrome II film to help monitor the changes in larval pigmentation patterns. Measurements were made using an ocular micrometer on either a Zeiss compound microscope or a Wild M-5 dissecting microscope.

Larval specimens for one micrometer sections were not relaxed prior to fixation. They were initially fixed for one-half hour to one hour, at room temperature, in a solution of 2.5% glutaraldehyde, 0.2 M phosphate buffer (pH = 7.6) (Millonig, 1961) and 0.34 M sodium chloride (Dunlap, 1966; Cloney and Florey, 1968). The specimens were then rinsed at room temperature in three, five minute rinses of 2.5% sodium bicarbonate solution (pH = 7.2). They were post-fixed for one-half hour to one hour at room temperature in a solution of 2% osmium tetroxide in 1.25% sodium bicarbonate (pH = 7.2) (Cloney and Florey, 1968). After fixation, the specimens were rinsed in distilled water and dehydrated through a graded series of ethanols. They were then placed in two changes of propylene oxide and embedded in LX-112 epoxy resin (Ladd Research Industries) following the method of Luft (1961).

One micrometer sections were cut on a Porter-Blum Sorvall MT-II ultramicrotome using glass knives. The sections were heat mounted on glass slides and stained with methylene blue and azure II (Richardson _et al._, 1960). To prevent wrinkling of the sections, the slides were dipped in a mixture of gelatin and K chrom-alum (V. Martin, personal communication) prior to mounting the sections on the slides. Sections were photographed on Kodak Pan-X film using a Zeiss photomicroscope II, or a Wild M-20 compound microscope.

To determine the histological changes at metamorphosis, competent larvae were induced to metamorphose using sand from the tubes of adult Sabellaria cementarium. One, two and three day post-settled juveniles were fixed using the methods described above for one micrometer sections.

Specimens for transmission electron microscopy (TEM) were fixed and embedded using the methods described for one micrometer sections. Sections were cut on a Porter-Blum Sorvall MT-IIB ultramicrotome with a diamond knife. Ribbons of silver-gold interference colors were mounted on bare copper grids. Sections were stained with saturated uranyl acetate (Watson, 1956) and lead citrate (Reynolds, 1963). Sections were examined and photographed on either a Phillips EM 200 or EM 201 electron microscope.

For histological staining, adult specimens were relaxed in isotonic (7.5%) magnesium chloride and fixed in Bouin's fluid. They were cut into pieces, dehydrated through a graded series of ethanols and vacuum embedded in Paraplast (melting point, 56-57°C). Serial sections were cut at 7 µm on an American Optical rotary microtome. The sections were stained with Harris' haematoxylin (Humason, 1967) and precipitated eosin. The sections were photographed on a Zeiss photomicroscope II using Kodak Pan-X film.

For scanning electron microscopy (SEM), specimens were relaxed in isotonic (7.5%) magnesium chloride in sea water. The specimens were placed in modified Beem capsules to which 53 µm Nitex mesh had been placed on both ends. This was found to be the best method for keeping the specimens clean. Specimens were either fixed as described above for one micrometer sections or they were fixed for four hours at room temperature in a solution of 2% osmium tetroxide in 1.25% sodium bicarbonate (pH = 7.2) (Cloney and Florey, 1968). After fixation, the specimens were rinsed in distilled water and dehydrated in a graded series of ethanols. They were then transferred through a graded series of iso-amyl acetate and critically point-dried for one hour in a Denton $CO_2$ critical point dryer. The specimens were mounted on aluminum stubs and sputter-coated with gold. They were then viewed and photographed on a Cambridge Stereoscan 150 electron microscope operating at 20 KV.

An experiment testing the effects of different types of substrates on the induction of metamorphosis in the competent larvae was carried out in the laboratory. The substrates tested were: beach sand from False Bay, San Juan Island, Washington and the tubes of the adult sabellariids *Idanthrysus ornamentatus*, *Phragmatopoma lapidosa*, and *Sabellaria cementarium*. The beach sand and the tubes were ground up using a mortar and pestle and air dried for at least three weeks prior to the start of the experiment. The sand from the sabellariid tubes included pieces of the tube cement. A control consisting of only Millepore prefiltered sea water was used. The different substrates used were placed in Pyrex custard cups with

100 ml of Millepore prefiltered sea water and algal cells at a concentration of $10^4$ cells per ml. Algae were added periodically throughout the experiment. Twenty-five larvae that had the morphological characteristics of competency were added to each of the dishes (thirty larvae were placed in the dish with P. lapidosa sand). After eighteen days the number of metamorphosed juveniles was recorded. Throughout the experiment frequent observations were made to characterize the searching behavior of the competent larvae. At the end of the experiment, the young juveniles were kept in the dishes to record their changes in morphology with age. They were periodically fed Dunaliella and Isochrysis in a 3:1 mixture at a concentration of $10^4$ cells per ml.

# RESULTS

## A. LARVAL DEVELOPMENT AND BEHAVIOR

Ripe Sabellaria cementarium of both sexes can be collected throughout the year from Griffin Bay, San Juan Island, Washington, by dredging. Not all worms bear viable gametes as one often finds non-motile sperm in the males, oocytes undergoing disintegration or worms that have already spawned. S. cementarium is a polytelic species, however, it appears that the population is capable of producing viable gametes throughout the year (Winesdorfer, 1967; personal observations).

The gamete-bearing abdominal segments of the sexually mature females are magenta in color, while those of the mature males are cream-colored. The spawned oocytes range from 80-85 µm in diameter. They are irregular in shape and possess a prominent germinal vesicle. Upon exposure to sea water for several minutes, the oocytes round up and their germinal vesicles break down (Fig. 3A). The nucleoli are dispersed throughout the oocytes. An ellipsoid vitelline membrane elevates from the surface of the oocyte and prominent cytoplasmic filaments are seen within the perivitelline space extending from the surface of the oocyte to the vitelline membrane. The changes in the oocytes described here agree with those described by Winesdorfer (1967) for S. cementarium and by Novikoff

(1937, 1939) for S. vulgaris. The sperm are of the primitive type as defined by Franzén (1956) and bear an acrosome 5 µm long. Their tails are ca. 50 µm in length.

Cleavage is spiral and holoblastic. The first polar body is extruded from the animal pole at 1.5 hours post-fertilization, followed by the extrusion of the second polar body at 2 hours post-fertilization (Fig. 3B). Just prior to the extrusion of the polar bodies there is a clearing in the cytoplasm at the animal pole. At first cleavage, 2.5 hours post-fertilization, a polar lobe is formed at the vegetal pole resulting in the trefoil stage (Fig. 3C). Approximately 15 minutes later, the polar lobe flows into the CD blastomere. This CD blastomere is much larger than the AB blastomere. At 4 hours post-fertilization, a second polar lobe which is much smaller than the first polar lobe is formed from the CD blastomere. At completion of the second division, this polar lobe flows back into the D blastomere. This division has produced three blastomeres (A,B,C) of approximately equal size and one much larger blastomere, D. During the third cleavage, 5.5 hours post-fertilization, a small polar lobe is formed from the D blastomere. This cleavage pattern results in the formation of the first quartet of micromeres.

The following description of larval development is based upon cultures raised in the laboratory at 10 -14°C. There was a great deal of asynchrony in the developmental rate among larvae within the same cultures and among larvae from different cultures. Therefore, the chronology of development presented here is representative of the fastest observed developmental rates and is summarized in Table 1. Planktonic development was divided into 10 stages based on the development of external characteristics. These 10 stages correspond to the stages sectioned to determine the histogenesis and organogenesis of the planktonic larvae.

### 18 Hour Pretrochophore

At 18 hours post-fertilization there is a swimming, negatively geotactic pretrochophore measuring 86 µm in diameter (Fig. 4A). It is irregular in shape and is surrounded by a prominent vitelline membrane. The pretrochophore appears opaque due to the magenta pigmentation and the large amount of yolk. The pretrochophore swims in an erratic tumbling manner at the water's surface in the culture dish.

### 23 Hour Trochophore

By 23 hours post-fertilization an early trochophore larva with a prominent prototroch and apical tuft, measuring 86-88 µm in diameter is seen (Fig. 4B, 7A). The prototroch divides the larva into two regions, an upper episphere and a lower hyposphere. The apical tuft is composed of a 8-10 long cilia which are approximately 48 µm in length. The apical cilia are often held erect in front of the swimming trochophore, however, they are not twisted together. The tuft is often seen to beat slowly from one side of the episphere to the other. Small yellow-green chromatophores are present on the sides of the episphere. The vitelline membrane is wrinkled in the regions of the apical tuft and hyposphere; it is closely applied to the surface of the trochophore and will eventually form the larval cuticle (Fig. 4B). A single gland pore is located directly below the prototroch on the dorsal surface (Fig. 7A). There are two tufts of cilia on the bottom of the hyposphere which appear as a single tuft under the light microscope (Fig. 7A). The long prototrochal cilia beat in a metachronal wave propelling the larva through the water. As the trochophore swims in a spiral path it rotates on its longitudinal axis.

### 44-46 Hour Trochophore

In the 44-46 hour trochophore a faint outline of the forming gut can be distinguished (Fig. 4C). The trochophore measures 90 µm in length and has traces of red and yellow-green chromatophores on the dorsal surface of the episphere. The prototrochal cilia have increased in length and number, as have the number of cilia in the apical tuft. The larvae swim randomly on a spiral path throughout the culture dish.

### 60-70 Hour Trochophore

Provisional setae first appear at 60-65 hours post-fertilization. There is a single seta, 9 µm in length, on each side of the hyposphere (Fig. 4D). These setae lack the serrated annulations found on the setae of older larvae. The apical tuft is composed of about 20 long cilia which are surrounded by several short cilia on either side. A second row of short cilia is beginning to appear in the prototroch below the first row of cilia.

By 70 hours a second pair of short provisional setae have formed and the primary pair now has 3-4 annulations per seta (Figs. 5A, 7B). The setae are capable of being flexed laterally to the sides of the body. The outline of the gut can be seen and using Nomarski microscopy, beating cilia can be distinguished for the first time within the stomach (Fig. 5A). Greenish lipid droplets are also observed in the stomach cells. The larva now measures 92 µm in length and yellow-green chromatophores are present on the episphere with patches of red chromatophores. There is a faint red eyespot located dorsally on the left side of the episphere, anterior to the prototroch.

### 3.5-4 Day Trochophore

The trochophore begins to feed after 3.5-4 days. It possesses 3-4 pairs of provisional setae and measures about 95 µm in length (Fig. 5B). A band of medial longitudinal cilia, the neurotroch, has appeared on the ventral surface of the hyposphere and extends from the mouth to the anal pore. The neurotrochal cilia beat away from the mouth. The body is still opaque and magenta in color but on the episphere are found yellow-green chromatophores extending over the dorsal surface. There are lipid droplets lying above the stomach and intestine. The single eyespot on the episphere is now prominent due to its increase in size. The apical tuft is composed of 3 ciliary bundles, 1 central bundle with 2 bundles of short cilia on either side of it. The vitelline membrane can no longer be distinguished (Fig. 5B).

### 5 Day Trochophore

The 5 day old trochophore possesses 5 pairs of provisional setae which are approximately 2.5 times the length of the body (Fig. 8C). The prototroch is composed of a prominent upper band of long cilia on the ventral surface which extends to the sides of the dorsal surface. The posterior band, now very prominent, is composed of shorter cilia (Fig. 8A). This band extends along the length of the now prominent oral hood fold where it meets the oral ciliation. The apical tuft is composed of 3 bundles of short cilia (Fig. 8B). The central bundle is non-motile while the other 2 bundles are motile.

The trochophore is beginning to lose its opaqueness and is becoming transparent (Fig. 5C). It is still magenta in color with patches of red and yellow-green chromatophores scattered over the surface of the episphere. Green lipid droplets are present on the dorsal surface of the larva. The trochophores of this stage swim randomly in a spiral fashion throughout the culture and do not exhibit any phototactic behavior.

## 12 Day Trochophore

The 12 day trochophore is characterized by the elongation of the hyposphere and the loss of the magenta pigmentation (Fig. 5D). The larva measures approximately 100 µm in length with 8-10 pairs of provisional setae present. The hyposphere has elongated but with no evidence of segmentation. Red and yellow-green chromatophores have appeared for the first time in this region. The green lipid droplets overlying the stomach and intestine are still present. The telotroch and grasping cilia have not formed yet.

On the dorsal surface of the episphere the yellow-green chromatophores have fused into large patches and the red chromatophores are scattered amongst them. There is still only one red eyespot present on the episphere (Fig. 5D). The margin of the oral hood fold is thickening and is a well-developed flap above the mouth. The neurotroch is now a well-developed band of cilia extending from the mouth to the anal pore on the ventral surface. The larvae swim randomly through the culture dish and exhibit a weak positive phototaxis.

## Metatrochophore

This stage is reached in about 18 days and measures approximately 240 µm in length (Figs. 6A, 9A). It is characterized by the division of the hyposphere into 2 segments and a pygidium. There are about 20 pairs of provisional setae present in the setal sacs. The apical tuft is composed of 3 bundles of short cilia which beat laterally. There are now 2 red eyespots located anteriorly to the prototroch on the dorsal surface of the episphere. Large patches of yellow-green and red chromatophores extend dorsally over the surface of the episphere and a band of black pigmentation lies directly posterior to the prototroch. Lip folds are forming on either side of the mouth posterior to the large oral hood fold (Fig. 9A). The upper band of prototrochal cilia runs along the anterior edge of the oral hood to the dorsal surface where there is a gap on the dorsal medial surface. The posterior band of the prototroch extends along the oral lips where it meets the oral ciliation. Lying between the two bands of cilia is the food groove.

Posterior to the prototroch, the hyposphere is divided into two segments by faint bands of black pigmentation on the dorsal surface of the hyposphere (Fig. 6A). Scattered over the stomach and intestine are green lipid droplets and red and yellow-green chromatophores. Anterior and lateral to the telotroch are the grasping cilia which appear by light microscopy to be fused. They undergo flicking motions in a group rather than the typical ciliary beating. Using SEM, the grasping cilia appear to be composed of cilia lying in close proximity to each other but which are not fused together (Fig. 9B). They function to hold the provisional setae to the sides of the body during swimming. The telotroch extends around the posterior end of the hyposphere, now called the pygidium (Fig. 9A). The telotroch splits on the dorsal surface so that the medial portion lacks cilia. Below the telotroch are large patches of reddish-black chromatophores. The larvae at this stage swim randomly throughout the culture bowl just above the bottom of the dish and are negatively phototactic.

### Metatrochophore with Tentacle Buds

Within 4-4.5 weeks post-fertilization, the larvae have developed tentacle buds. The body is divided into the episphere, 3 parathoracic segments, 3 faint abdominal segments, and a pygidium (Figs. 6B, 9C).

The apical tuft is now composed of 5 bundles of cilia. There are 4 red eyespots on the dorsal surface of the episphere anterior to the prototroch. The yellow-green chromatophores are gradually being replaced by the reddish-black chromatophores with a few scattered patches of green chromatophores amongst them. Located posterior to the eyespots on the dorsal surface of the episphere are two tentacle buds which are very short and bear a few red pigment spots on their dorsal surfaces (Figs. 6B, 9C). On the ventral surfaces of these tentacle buds are developing ciliary tracts. The two prototrochal bands are located on the posterior margin of the episphere and they separate the episphere from the trunk segments. There is a gap in the prototroch on the dorsal surface between the tentacles where the dorsal hump is now located (Fig. 9C). A band of black pigment separates the prototroch from the trunk.

Located within the setal sacs are 25-30 pairs of provisional setae (Fig. 9C). The elongated trunk is divided into 3 parathoracic segments by black pigment bands which extend across the dorsal surface of the larva (Fig. 6B). Posterior to the parathoracic segments are 3 faintly demarcated abdominal segments (Fig. 6B). There are no setae or uncini present on the trunk segments. On the pygidium are located the grasping cilia and the telotroch. Posterior to the telotroch are patches of reddish-black chromatophores (Fig. 6B).

The larvae are photopositive and swim over the bottom of the culture bowl in a spiral path. Sometimes, the larvae were seen to swim in loops directly above the bottom of the bowl. When this behavior was observed, the larvae did not rotate on their longitudinal axis in a spiral path. The larvae would apply the top of the episphere to the bottom of the culture dish when exhibiting this behavior pattern.

## Nectochaeta (Competent Larva)

This stage represents the fully developed planktonic or competent larva and occurs 5.5-6 weeks post-fertilization. It measures approximately 350 µm in length. The body is divided into 5 distinct regions: the episphere, one asetigerous thoracic segment, 3 setigerous parathoracic segments, 3 abdominal segments with uncinigerous lobes, and the pygidium (Figs. 6C, 10A,C).

The episphere possesses 4 reddish-black eyespots on its dorsal surface. The posterior pair of eyespots is situated closer together than is the anterior pair. Scattered over the dorsal surface of the episphere are patches of reddish-orange chromatophores. The yellow-green patches of pigment observed previously have completely disappeared. Small patches of green chromatophores lie directly anterior to the prototroch on the dorsal surface. The prototroch is situated around the posterior margin of the episphere except for a gap on the dorsal surface between the tentacles. There are 5 tufts of apical sensory cilia and numerous raised gland pores on the apical end of the episphere (Figs. 14A,B).

The tentacles arise from the dorsal surface of the episphere and lie posterior and lateral to the eyespots. The tentacles extend approximately one-half the length of the body of the larva. Located on the dorsal surface of the tentacles are patches of reddish-black chromatophores, raised gland pores, and ciliated sensory tufts (Figs. 11B, 13B). The tips of the tentacles are heavily ciliated as are the ventral surfaces which bear a ciliated band running its entire length (Fig. 11A). The dorsal hump lies between the tentacles (Fig. 10C). Situated below the prototroch and postero-lateral to the tentacles are the opercular cirri (Fig. 11C). The cirri will form the fleshy papulae located below the outer opercular paleae in the adult worms. The junction between the cirri and the prototroch is demarcated by a band of black chromatophores.

There are approximately 40 pairs of provisional setae within the setal sacs (Fig. 12B). Hidden amongst the provisional setae are the short primary settling paleae which will form the operculum of the newly metamorphosed juvenile. On the dorsal surface of the larva, nototrochal cilia extend from the posterior portion of the first parathoracic segment to the posterior portion of the third parathoracic segment (Fig. 12C). Scattered black chromatophores are found on the dorsal surface of the parathoracic and the abdominal segments. Between each segment is located a distinct band of black and green chromatophores. The parathoracic segments each bear one pair of winged capillary setae and one pair of curved capillary setae in their parapodial lobes (Fig. 12C). Each of the abdominal segments has two large uncinigerous lobes bearing uncini (Fig. 12A). The number of uncini per lobe varies among specimens but there are never more than 8 uncini per lobe. On the ventral surfaces of the parathoracic segments are found the ciliated sensory tufts and recessed gland pores (Fig. 13A). On the ventral surface of the thoracic segment, directly below the oral lip folds is found the building organ (Fig. 11C). The neurotroch extends from the mouth to the telotroch on the medial ventral surface

(Fig. 10B).

The pygidium possesses the telotroch and the grasping cilia. Scattered patches of reddish-black chromatophores are found below the prototroch on the pygidium. Raised gland pores surround the anus and scattered sensory tufts are found amongst the gland pores (Fig. 14C).

The competent larvae are positively phototactic and positively geotactic. When a strong light is shone on the side of the culture dish, the larvae will aggregate on the bottom of the dish closest to the light source. At this point the larvae search for a site on which to metamorphose by exploring the sand grains on the bottom of the culture vessel.

The larvae swim directly above the substrate and often were seen to hover in an erect position over the substrate. It was noted that groups of 5 to 6 larvae tended to remain in close proximity to each other while exploring the substrate. The larvae stop swimming and crawl over the substrate, applying the dorsal surface of the tentacles to the substrate. As described earlier, the larval tentacles bear ciliated sensory tufts. While crawling the larva uses both the telotroch and the prototroch for locomotion. The larva stops crawling and applies the building organ, bearing sensory tufts, directly to the sand grains (Fig. 15B). To do this, the oral hood fold is bent forward so that the surface of the building organ is fully applied to the sand grains. It has been noted that the larva will ingest sand grains when the oral hood is applied to the sand grains. The prototrochal cilia of the oral hood fold sweep small sand particles into the mouth and the cilia of the neurotroch rejects them posteriorly. These same sand particles will be swept into the mouth and rejected several times before they are finally ingested by the larva. The sand particles can be traced moving through the stomach and intestine of the larva and they can be found in the fecal pellets. After several minutes the larva will resume swimming over the bottom of the bowl.

Larvae were also noted to apply the tops of their epispheres directly to the sand grains. The larva will remain stationary in an upside down position and will rotate on its central axis (Fig. 15A). The episphere is applied to the sand grains the entire time. This behavior lasts several seconds after which the larva falls on its side and explores the substrate with either its tentacles or its building organ. Several seconds later the larva will get up and repeat this behavior. The larvae were often seen to swim in an upside down position bouncing their apical ends over the substrate.

Larvae were observed to bury themselves underneath and between sand grains. Once between the sand grains the larvae would cling tenaciously to them. It would take several forceful squirts of water from a pipette to dislodge them. As the larvae moved between the sand grains, they would use their provisional setae to push the sand grains aside so that they could move freely between them. After several minutes the larvae would resume swimming over the bottom.

## Provisional Setae and Defensive Behavior

The provisional setae first appear in the 60-65 hour trochophore and are present throughout the life of the larva. The setae are secreted in the posterior facing setal sacs located on either side of the body at the level of the prototroch. Provisional setae are continuously added throughout the life of the larva until about 40 pairs are present in the competent larva. They reach a length of up to 300 µm. Along their length at approximately 8 µm intervals are annulations (Fig. 12B). Situated around the perimeter of these annulations are numerous tooth-like serrations.

The larvae are capable of spreading their provisional setae so that they completely surround the body and point in all directions (Figs. 16A,B). This behavior appears with the first setae and continues throughout the larval life. The larvae spread their setae in response to irritation or if they make contact with another larva. In cultures, the creation of vibrations in the water would elicit this response and the larva did not have to be touched directly. Competent larvae used their setae to move sand grains out of the way when they were crawling over and between the sand grains.

To flex the setae, the larva constricts the prototrochal region and the anterior end by the contraction of the prototrochal muscle and the esophageal muscles which connect to the anterior region and setal sac muscles. With the contraction of the muscles surrounding the setal sacs, the setal sacs are turned to the side and face laterally. The setae then spread out so that they surround the body. The setae stay erect for several seconds and then they relax. If the irritation continues, the setae will continue to be spread and relaxed until the irritation stops.

## Feeding Behavior

The capture of food particles was not observed in the trochophore larvae of S. cementarium, however, it was observed in the metatrochophores and competent larvae. The method of particle capture in the trochophores was inferred from these late stage larvae. The larvae of S. cementarium feed by an opposed ciliary band system. The upper and lower ciliary bands of the prototroch appear to beat in opposition to each other with the lower band acting as a metatroch. The longer cilia of the upper prototrochal band capture the food particles and carry them towards the lower band of cilia. The lower band carries the food particles into the food groove. The cilia of the food groove carry the particles to the mouth where they are swept into the mouth by the oral ciliation.

When the larvae encounter particles too high in concentration, they will stop feeding. They no longer filter the particles due to the fact that the posterior band of prototrochal cilia ceases to beat. This is the same mechanism as that observed by Strathmann _et_ _al_. (1972) for _Spirabranchus_ trochophores. If the larvae encounter particles that are too large to handle they will reject them. Strathmann _et_ _al_. (1972) have shown the _Spirabranchus_ trochophores use their neurotrochs to carry food particles away from the mouth. Observations on the larvae of _S_. _cementarium_ have found that they too use the neurotroch to reject particles that are too large to ingest. The particles are carried posteriorly along the hyposphere of the trochophores or along the trunk segments of the late larvae by the posterior beat of the neurotrochal cilia. Once inside the mouth the particles are carried into the esophagus by the esophageal ciliation. As the particles are carried down the length of the esophagus, they are rotated in a clockwise direction by the cilia of the esophagus and then swept into the stomach. In the stomach, the food particles are formed into a bolus which is rotated in a counter-clockwise direction by the cilia of the stomach. The bolus is forced into the intestine by the muscular contraction of both the stomach and the body wall of the larva. The bolus is broken down in the intestine by the cilia and it is defecated through the anal opening.

## B. ORGANOGENESIS AND HISTOGENESIS OF THE PLANKTONIC LARVAE

The organogenesis and histogenesis of the planktonic larvae of Sabellaria cementarium were studied by examination of histological sections of 10 developmental stages. The external morphologies of these stages have been described in the preceding chapter. The larval structures to be examined are as follows: body wall, muscle system, coelom, alimentary tract, nervous system, circulatory system, gland cells, and tentacles. The 18 hour pretrochophore will be described separately as it is not encompassed in the development of any of the larval structures.

The 18 hour pretrochophore appears to lack a blastocoel (Fig. 26A). Lipid droplets are abundant and they obscure the underlying cells. There are apparently two types of lipid droplets; those which stain green and those which stain blue (Richardson's stain) (Fig. 26A). Cell types cannot be distinguished at this time except for a presumptive mucoid gland cell which appears as a pale-staining cell located in the region of the developing episphere (Fig. 26A). Cilia as well as presumptive prototrochal cells cannot be detected by light microscopy. The larva does, however, swim in an erratic tumbling manner. Although the vitelline membrane is prominent externally, it does not stain in one micrometer sections.

## Body Wall

The following structures were examined in the body wall of the larvae: cuticle, epidermis, and setal sacs. Body wall musculature and peritoneum will be described in the sections on the muscle system and coelom, respectively.

### i) Cuticle

The vitelline membrane or egg envelope is retained as the cuticle in the early larvae. It completely surrounds the larva and appears to be composed of two zones. The zones will be named according to the terminology of Eckelbarger and Chia (1978). Zone II is a thick, outer electron-dense layer consisting of closely packed fibrils (Fig. 17A). Lying directly below this layer and against the epidermis is Zone I. This zone is weakly-staining and is composed of loose fibrils (Fig. 17A). Branching microvilli can be seen extending from the surfaces of the epidermal cells through the vitelline membrane (Fig. 17A).

By 5 days, the vitelline membrane is being replaced by a thin cuticle secreted by the epidermal cells. In one micrometer sections, the surface of the cuticle stains lightly, while the cuticle itself is non-staining. In TEM sections, the newly formed cuticle appears electron opaque and there is a reduction in the number of microvilli extending through the cuticle (Fig. 17B).

### ii) Epidermis

The epidermis is a simple epithelium. In the early developmental stages, the cells are cuboidal in shape and contain a centrally located nucleus (Figs. 26A,B,D, 27A-D). As the larvae continue to grow, the cells tend to flatten particularly in the region of the hyposphere (Figs. 37C,D, 38A). Initially there are numerous lipid droplets located within the epidermal cells, however, these droplets decrease in number as the larvae grow and by 12 days the droplets are nearly exhausted. Pigment cells become apparent in the 12 day trochophore. These cells contain numerous black granules in their apical cytoplasm and possess basally located nuclei. The distribution of these cells within the epidermis corresponds to the external pigmentation pattern of the larva. Located within the epidermis are numerous gland cells which will be described in the section on gland cells.

The multiciliated prototrochal and telotrochal cells are cuboidal shaped with basally located nuclei (Fig. 18A). They bear cilia anchored by long primary rootlets and shorter secondary rootlets (Fig. 18A). Within the cytoplasm of these cells are numerous mitochondria.

### iii) Setal Sacs

The setal sacs containing the provisional setae are first apparent in the 46 hour trochophore. They appear as small spherical sacs located on either side of the body and contain 2 chaetoblast cells, each with a prominent nucleus (Fig. 19A). A single rudimentary seta can be seen developing within the sac. In the 65 hour trochophore the setal sacs have increased in size and the provisional setae are apparent externally (Fig. 19B).

Throughout the life of the larva, the setal sacs continue to increase in size due to an increase in the number of cells located within the sacs. The chaetoblast cells lie basally within the setal sacs and possess numerous microvilli (Figs. 20, 21B). The provisional setae are formed on the microvilli. Surrounding the chaetoblast cells are the lateral accessory cells which line the follicle in which the provisional seta lies (Figs. 20, 21B). The lateral cells secrete the cuticle which lines the follicle. The number of provisional setae secreted by a setal sac may approach 40 in the competent larva. The setal sacs are also responsible for the secretion of the primary opercular paleae found in the competent larva and newly metamorphosed juveniles. Large vacuoles begin to appear laterally and basally within the sacs in the 5 day trochophore and continue to increase in size and number throughout the larval life (Figs. 19D, 20). The function of these large vacuoles is unknown. The provisional setae are composed of chitin and in histological and TEM sections, numerous parallel longitudinal channels can be detected extending the entire length of the setae (Fig. 21B).

Located on the dorsal surface of the metatrochophore with tentacle buds are the developing parapodial and uncinigerous lobes. Within the cavities of these lobes are clusters of undifferentiated cells which, presumably, will give rise to the chaetoblast cells (Fig. 19C). These cells will secrete the parapodial setae and uncini. In the competent larva, the chaetoblast cells can be detected basally within the parapodial lobes (Fig. 19D). Lateral accessory cells can be seen lining the follicles in which the setae lie. Within the uncinigerous lobes, the chaetoblast cells are found at the apical ends of the lobes. The muscles associated with these lobes are described in the section on the muscle system as are the muscles of the setal sacs.

### Muscle System

Muscles can first be identified in one micrometer sections of the 3.5 day trochophore. They appear as thin bundles of muscle fibers located on the lateral and basal surfaces of the setal sacs (Fig. 22A). These muscles are referred to as the setal sac muscles. They connect to a thin band of muscle situated anteriorly to the setal sacs, the prototrochal muscle (Fig. 22B). The prototrochal muscle extends around the circumference of the larva directly below the prototrochal cells. There is a medial branch of this muscle located ventrally at the level of the developing vacuolated stomach cells, which extends across the larva to connect to the prototrochal cells and to the setal sac musculature.

In the 5 day trochophore there is a thin band of muscle fibers arising from the apical end of the esophagus which extends along the lateral surfaces of the esophagus to insert on the medial branch of the prototrochal muscle (Fig. 22C). The medial branch of the prototrochal muscle inserts into the setal sac muscles which have undergone hypertrophy (Figs. 22C, 25A). The setal sac muscles now form a muscular capsule around the internal surfaces of the setal sacs (Figs. 20, 25A). Extending dorsally below the epidermis is a branch of the setal sac musculature which appears to insert onto the epidermis at the posterior end of the hyposphere. This appears to be a developing branch of the dorsal longitudinal muscle which is present in the metatrochophore with tentacle buds.

There is continued hypertrophy of the larval musculature in the 12 day trochophore and metatrochophore. In the metatrochophore with tentacle buds, situated above the esophagus, is the supraesophageal muscle which consists of approximately 2 parallel branches (Figs. 22D, 23A). These branches extend across the antero-ventral surface of the episphere to insert onto the lateral epithelium of the episphere. Arising from the supraesophageal muscle are the longitudinal muscles. These muscles are composed of approximately 5 bundles of muscle fibers which extend along the lateral surfaces of the esophagus. Located within the mesenteries associated with these muscles are the circumesophageal blood vessels (Fig. 40A). Also arising from the supraesophageal muscle are the lateral muscles of the episphere which lie below the lateral epithelium of the episphere to insert onto the setal sac musculature (Fig. 23B). The setal sac muscles are composed of numerous bundles of muscle fibers which form a capsule around the sacs (Figs. 22D, 23A,C). The proximal ends of the provisional setae appear to insert into the setal sac muscles. Arising from the setal sac muscles are muscles which insert ventrally onto the longitudinal muscles and medially onto the sides of the esophagus (Fig. 23A). Extending from the prototrochal cells is the medial branch of the prototrochal muscle which inserts onto the sides of the esophagus posterior to the setal sac branch (Fig. 23C). The longitudinal muscles become progressively thinner as they come to lie below the epidermis in the region of the trunk. This is due to the fewer number of muscle fibers present in this area. Circular muscles of the body wall cannot be detected at this time.

In the competent larva, the larval musculature is fully developed and there has been considerable hypertrophy of the elements comprising the muscle system. The supraesophageal muscle consists of two large bundles of muscle fibers located medially within the episphere (Fig. 23D). Arising from this muscle are the longitudinal muscles and the lateral epispheral muscles (Fig. 23D). The longitudinal muscles extend laterally along the sides of the esophagus and come to lie below the epidermis in the trunk region (Figs. 23D, 38C). The number of bundles of muscle fibers comprising the longitudinal muscles has increased and these muscles now give off branches into the parapodia of the parathoracic region and into the uncinigerous lobes of the abdominal segments. In cross section, the muscle bundles of the dorsal longitudinal muscles appear to be larger than those of the ventral muscles (Fig. 24C). Arising from the prototrochal cells in the episphere are the muscle bundles of the medial prototrochal muscle which extend to the sides of the esophagus in the region of the esophageal-stomach junction (Fig. 24B). Projecting from the posterior surface of the setal sacs are muscle bundles that extend medially across the larva to the longitudinal muscles and to the sides of the esophagus in the region of the esophageal-stomach junction (Fig. 24A). Arising from these medially located muscles are branches that extend onto the dorsal and ventral surfaces of the stomach in the region of the subesophageal ganglion. The circular muscles can be detected as a thin band of muscle lying between the epidermis and the longitudinal muscles in the trunk region (Fig. 24D). The muscles of the tentacles will be described in the section on the larval tentacles.

## Coelom

The coelom appears to be formed between 3.5 and 5 days by a splitting of the lateral mesoderm (Fig. 25A). A peritoneal membrane is not evident at the light microscopic level; however, TEM observations on the 5 day trochophore show the presence of a thin peritoneal membrane lining the coelomic cavity (Fig. 25B). This coelomic cavity extends from the most anterior portion of the episphere to the end of the hyposphere. Concentrated at the posterior end of the hyposphere are the presumptive mesodermal cells (Figs. 22C, 25A). These cells may be involved in the formation of the segmental coelomic cavities in the later larval stages. These cells appear to be present throughout larval life.

The coelom consists of a single cavity until the metatrochophore with tentacle buds. At this stage segmental coelomic cavities, corresponding to the external segmentation of the larva, appear to be forming (Fig. 25C). The coelom thus consists of an anterior coelomic cavity and the segmental coelomic cavities separated by thin segmental septa (Fig. 25D). Within the competent larva, it is difficult to distinguish the coelomic cavities due to the enlargement of the vacuolated cells of the stomach and the presence of the parathoracic gland cells. Coelomocytes are not observed within the coelomic cavities of any larval stage.

## Alimentary Tract

The method and timing of gastrulation cannot clearly be determined using one micrometer sections. As there is no invagination occurring, it would appear that gastrulation occurs by an inward epibolic movement of the presumptive endodermal cells. This method of gastrulation has previously been reported for those polychaete species in which the blastula contains a few large, yolky, presumptive midgut cells and no blastocoel (Anderson, 1966). Gastrulation may have occurred prior to the 18 hour pretrochophore as the presumptive midgut and stomadeal cells cannot be distinguished.

The presumptive endodermal cells apparently divide to give rise to the esophagus and stomach of the larva. A small indentation which may represent the developing stomodeal invagination can be seen postero-ventrally in the 18 hour pretrochophore (Fig. 26A).

The developing alimentary tract can first be distinguished internally in the 23 hour trochophore. The developing esophagus appears as an anteriorly located ring of cells surrounding a ciliated lumen (Fig. 26B). Lying adjacent to the esophagus are the ectodermal cells and the mesodermal cells. The cells of the esophagus are irregular to cuboidal in shape and stain-lightly with Richardson's stain. Each of the cells bears several cilia which extend into the esophageal lumen. A thin cuticle lines the surface of the lumen. One of the postero-lateral cells of the esophagus is much larger than the other cells of the esophagus and extends into the region of the developing stomach. The stomach appears as a thin lumen developing within the cells of the hyposphere (Fig. 26B). These presumptive stomach cells appear to be undergoing ciliogenesis. A cuticle is not evident within the region of the developing stomach. The cells of the stomach contain two types of lipid droplets as seen in the 18 hour pretrochophore.

By 46 hours, the developing gut can be observed externally. The esophagus consists of ciliated columnar cells which appear to be connected to the stomodeal invagination which has now become ciliated (Fig. 26C). Lipid droplets appear to be concentrated in the anterior esophageal and ectodermal cells (Fig. 26D). The cilia of the anterior esophageal cells are directed posteriorly into the esophageal lumen while those of the posterior cells are directed anteriorly (Fig. 26D). The large postero-lateral cell of the esophagus described in the 23 hour trochophore now contains several small, spherical vacuoles. The stomach is composed of columnar cells and is continuous with the esophagus. These cells bear only a few cilia and the lumen lacks a cuticular lining (Fig. 26D).

In the 65 hour trochophore, the columnar esophageal cells are heavily ciliated and the cilia densely pack the lumen of the esophagus (Fig. 27A). Lipid droplets are abundant in the anterior esophageal cells and in the junctional area between the esophagus and the ventral surface of the stomach (Fig. 27C). The single, large vacuolated cell found in the postero-lateral portion of the esophagus is located in the ventral region of the junction, between the esophagus and the stomach (Fig. 27B). The stomach cells are columnar in shape and possess few cilia (Fig. 27C).

By 3.5-4 days, the trochophore has begun to feed and the anus can now be detected in one micrometer sections. The alimentary tract is divided into the esophagus and a prominent stomach (Fig. 27D). There does not appear to be a demarcation between the stomach and the intestine at this time. The esophagus has increased in length and consists of columnar cells laterally and flattened cells anteriorly (Fig. 27D). The cells are multiciliated and possess pale-staining nuclei and cytoplasm. The cilia project into the lumen and form a large central swirl. There is a thick cuticular lining of the esophageal lumen and microvilli extend from the surfaces of the cells through the cuticle (Fig. 17B). These microvilli persist through the adult stages. Lipid droplets are concentrated in the anterior esophageal and ectodermal cells and in the junction between the stomach and the esophagus. Located ventrally in this junction are several large cells extending from the postero-lateral surfaces of the esophagus. These cells contain large pale-staining nuclei and numerous spherical vacuoles (Fig. 36D). These cells are derived from the postero-lateral esophageal cell in the 23 hour trochophore. They continue to increase in size and number throughout the larval life. The cells of the stomach are thick anteriorly and multiciliated (Fig. 36D). In the posterior portion of the stomach the cells are thin and flattened. The cells located in the dorsal regions of the stomach are lightly ciliated, while those in the ventral regions are heavily ciliated (Fig. 36D).

In the 5 day trochophore, the stomach and intestine are demarcated by a septum which extends from the ventral wall of the alimentary tract (Fig. 28A). The septum consists of several flattened, weakly ciliated cells. Ventrally and laterally, the stomach is composed of thin, flattened, cells along its entire length. The vacuolated cells now contain a blue-staining flocculent material within their spherical vacuoles and possess a border of cilia and microvilli on their luminal surfaces (Fig. 30A,B). The concentration of lipid droplets above the esophagus and in the region of the vacuolated cells has decreased so that only a few scattered droplets remain (Fig. 28A). The walls of the intestine consist of thin, flattened, cells with pale-staining nuclei and cytoplasm. These cells appear more heavily ciliated than those found in the lower stomach.

The stomach and intestine are highly differentiated in the metatrochophore, while the esophagus remains little changed. At the junction between the esophagus and the stomach, located within the lumen is a dense border of cilia (Fig. 28B). These cilia extend into the lumen from the posterior esophageal cells. Ventrally and anteriorly, the stomach consists of large, thick, vacuolated cells which contain blue-staining flocculent material within the spherical vacuoles (Fig. 28C). Each of these cells possesses several large vacuoles surrounded by numerous smaller vacuoles. These smaller vacuoles appear to coalesce to form the larger vacuoles. Dorsally the stomach is comprised of flattened, weakly ciliated cells which contain large green-staining lipid droplets (Fig. 28C). Also found within these cells are smaller, clear spherical vacuoles which contain irregularly shaped greenish-blue-staining granules (Fig. 28D). These vacuoles may contain zymogen granules. Similar cells are found on the ventral surfaces of the stomach and in the septum dividing the stomach and the intestine. The intestinal cells are much thinner on the dorsal surface of the intestine than on the ventral surface. They contain a few lipid droplets and phagocytic vacuoles (Fig. 37D). The intestinal cells have pale-staining nuclei and cytoplasm and are much more heavily ciliated than those of the stomach (Fig. 37D).

Throughout the development of the larva, the esophagus, stomach and intestine continue to grow. In the metatrochophore with tentacle buds, gland cells are beginning to form in the lateral surfaces of the esophagus. A pair of cuboidal cells containing granules which stain pinkish-purple have appeared (Fig. 29B). These cells apparently are precursors of the esophageal glands found in the competent larva. Two types of cells, other than gland cells, can be distinguished within the esophageal epithelium. The first type which lines the lumen contains a pale-staining nucleus and cytoplasm (Fig. 29A). The second type of cell is found laterally and contains a dark-staining nucleus and cytoplasm (Fig. 29A). The vacuolated cells of the ventral and anterior portions of the stomach continue to increase in size due to an increase in the number of vacuoles within each cell. The nuclei of these cells now stain dark blue instead of pale blue as seen in the earlier stages.

The esophagus of the competent larva contains 3 types of gland cells. Situated laterally and anteriorly, are the cuboidal cells which contain pink granules (Fig. 38D). Lying ventrally on the apical surface of the esophagus and in the region of the oral hood fold are gland cells, irregular in shape, which contain a pink flocculent material in which are found numerous blue granules (Fig. 29C). The lumen of the esophagus is heavily ciliated and the epithelium is composed of 2 cell types as in the metatrochophore with tentacle buds (Fig. 29C).

The anterior and lateral portions of the stomach are surrounded by the vacuolated cells which extend along the ventral surface of the stomach (Fig. 29D). These cells continue to increase in size by the multiplication of the spherical vacuoles contained within. These cells bear prominent cilia which extend into the lumen of the stomach. Within a short distance of these vacuolated cells the stomach consists of thin, flattened, epithelial cells. These cells are weakly ciliated and contain pale-staining nuclei and cytoplasm which contains numerous small blue granules which are presumptive zymogen granules. The stomach cells also contain lipid droplets (Fig. 33D).

The intestine is composed of flattened cells with pale-staining nuclei and cytoplasm (Fig. 29D). Within these cells are a few large lipid droplets. The intestinal cells are more heavily ciliated than those of the lower stomach particularly in the region of the anus.

## Nervous System

The fully developed larval nervous system consists of a cerebral ganglion, circumesophageal commissures, ventral ganglia, a subesophageal ganglion and paired ventral nerve cords.

The cerebral ganglion, or brain, first appears in the 12 day trochophore larva. It is located dorsally in the episphere directly above the esophagus and lies in close association with the epidermal cells. The neuropile of the ganglion can be distinguished as a thin band of cytoplasmic filaments which lie ventrally to the nuclei of the nerve cell bodies and the apical epidermal cells (Fig. 31A). The nuclei of the nerve cell bodies and the apical epidermal cells cannot be distinguished from one another at this point. Processes of the neuropile extend amongst the nuclei to lie directly below the apical epithelium.

In the metatrochophore the neuropile has increased in size due to an increase in the nerve fibers and extends along the sides of the episphere to the level of the peripheral mucoid glands (Fig. 31C). There are a few scattered nuclei embedded within the neuropile. It is now bilobed and it is surrounded by a layer of nerve cell bodies on the anterior surface. The eyespots are developing by the invagination of the ectoderm to form pigment cup photoreceptors, however, they are not embedded within the cerebral ganglion but lie peripherally to it on either side of the episphere (Fig. 31D). The eyespots are surrounded by the apical epidermal cells and no distinct optic nerves can be detected connecting them to the ganglion at this stage.

Lying on the ventral surface of the esophagus are the dorsal roots of the circumesophageal commissure (Fig. 31B). They appear as thin, naked nerve processes lying on either side of the esophagus. The commissures do not appear to be connected to the cerebral ganglion. Distally they connect into the spheroid ventral ganglia which lie posteroventral to the esophagus. The ventral ganglia are composed of a central neuropile surrounded by a few scattered nerve cell bodies. Lying within the neuropiles are purple-staining granules of unknown function. The ventral roots of the circumesophageal commissure lie below the epidermis and extend from the region of the prototroch to the ventral ganglia (Fig. 31D). They are thin unsheathed tracts of nerve fibers which do not connect to the cerebral ganglion. The subesophageal ganglion lies ventrally between the esophagus and stomach and connects the two ventral ganglia (Fig. 31B). It is composed of a central neuropile surrounded by a thick layer of nerve cell bodies.

In the metatrochophore with tentacle buds and the competent larva there is considerable enlargement of the cerebral ganglion due to an increase in the number of nerve fibers in the neuropile (Figs. 32A,D, 33B). The eyespots are well defined with the pigment granules organized into a concave cup which is directed antero-dorsally. The eyespots are embedded within the nerve cell bodies of the cerebral ganglion and thin strands of nerve fibers can be detected running into the base of the eyespots (Fig. 33A). Similar fibers can be seen running to the apical sensory cilia (Fig. 33A).

The dorsal and ventral roots of the circumesophageal commissures are now connected antero-ventrally by thin processes which run along the muscles of the episphere (Figs. 32A, 33B,C). The subesophageal ganglion has enlarged and running posteriorly from the ventral ganglia are the ventral nerve cords (Figs. 32A,B, 33C). They lie on either side of the neurotroch and consist of a central neuropile surrounded by nerve cell bodies (Fig. 33D).

## Circulatory System

The fully developed larval circulatory system consists of a supraesophageal vessel, circumesophageal vessels, a dorsal blood vessel leading to a dorsal blood sinus, and a ventral vessel leading into a ventral blood sinus.

Forming blood vessels of the circulatory system are seen in the metatrochophore. The supraesophageal vessel appears in the episphere dorsally as a small vessel lying directly posterior to the cerebral ganglion (Fig. 34A). It consists of a small lumen surrounded by the apposed surfaces of the supraesophageal mesentery. The two circumesophageal vessels appear as small lumen within the mesenteries extending from the sides of the esophagus (Fig. 34A). They originate by a separation of the apposed surfaces of the mesenteries (Fig. 35A). Associated with the mesenteries which form the blood vessels are 1 to 2 prominent nuclei which face away from the lumen of the vessels (Fig. 35A). This positioning of the nuclei is common to all vessels derived from the mesenteries in this and later stage larvae.

In the metatrochophore with tentacle buds and the competent larva, the supraesophageal and circumesophageal vessels are very prominent. The supraesophageal vessel is composed of a large lumen surrounded by a thin membrane derived from the supraesophageal mesentery (Fig. 34B). This vessel extends over the dorsal surface of the cerebral ganglion and appears to connect to the posterodorsal surface of the cerebral ganglion and the dorsal blood vessel. The blood vessels of the tentacles will be described in the section on the tentacles. The circumesophageal vessels are found within the mesenteries which extend from the supraesophageal muscle to the sides of the esophagus (Figs. 22D, 40A). On the medial-lateral surface of the esophagus, each of the circumesophageal vessels contains 2 lumena lying in apposition to each other, while on the dorso-lateral and dorso-ventral surfaces of the esophagus they are composed of a single lumen. It thus appears that the circumesophageal vessels branch on the medial-lateral surfaces of the esophagus. The circumesophageal vessels do not appear to connect to the dorsal blood vessel.

The dorsal blood vessel is now present and extends along the dorsal surface of the esophagus and appears to arise as a lumen within the dorsal mesentery (Fig. 34C). The dorsal blood vessel runs into the dorsal blood sinus which extends dorso-laterally over the entire length of the gut (Fig. 34D). It originates by a separation of the mesodermal epithelium from the dorsal and lateral walls of the gut and consists of a large lumen surrounded by the mesodermal epithelium (Fig. 35B). The ventral blood vessel is found on the ventro-medial surface of the posterior portion of the esophagus and the anterior portion of the stomach. It consists of a small lumen which was probably derived from a ventral mesentery, as it is surrounded by a peritoneum extending from the ventral surface of the body wall. The ventral blood vessel appears to extend into a large ventral blood sinus which extends over the ventro-medial surface of the gut to the ventro-lateral surfaces (Fig. 34D). This blood sinus appears to be derived from the ventral mesodermal epithelium of the gut. Within all blood vessels and sinuses is found an acellular plasma.

## Gland Cells

The gland cells include the epispheral and pygidial mucoid glands and the parathoracic glands.

A unicellular, epidermal mucoid gland is first observed in the 23 hour trochophore (Fig. 36A). By 46 hours there is a pair of small unicellular mucoid glands situated in the episphere above the prototrochal cells. Each gland cell is spherical in shape with a large posteriorly located nucleus. Within the cytoplasm are numerous lipid droplets and a large, pink-staining (Richardson's stain) reticulate material. In the 65 hour trochophore, the cells have increased in size, are more spherical in shape, and are beginning to lose their lipid droplets. A pore is present in each cell which extends from the apex of the vacuole to the surface of the cuticle (Figs. 27A, 36B).

A third unicellular epidermal mucoid gland arises on the antero-ventral surface of the episphere in the 3.5 day trochophore (Fig. 36C). It is larger than the 2 lateral mucoid glands and appears as a laterally compressed cell extending across the apical surface of the episphere (Fig. 36C,D). The cell is covered by a thin epithelium on the apical surface and contains a small postero-laterally located nucleus. The pink-staining reticulate material is located within a large thin-walled vacuole. At the fine structural level, the mucoid secretion is composed of a fibrillar material (Fig. 39A,B).

In the 12 day trochophore, the mucoid glands of the antero-lateral surfaces of the episphere appear as either flattened cells, small irregular shaped spheres, or as large spherical cells (Fig. 37A,B). The nuclei of these unicellular mucoid cells are small and are usually laterally displaced. The mucoid material is contained within large vacuoles surrounded by thin walls. Pores can be detected which connect the vacuoles to the outer surfaces of the episphere (Fig. 37A). Mucoid glands are apparent in the postero-ventral portion of the elongating hyposphere (Fig. 37B). These glands appear morphologically identical to the mucoid glands of the episphere.

The unicellular mucoid glands extend over the entire ventral and lateral surfaces of the episphere in the metatrochophore (Fig. 37C). They lie adjacent to one another and are the major cell type comprising the epithelium of the episphere. These unicellular glands are spherical to irregular in shape with basally located nuclei and large vacuoles containing the reticulate mucoid substance. The vacuoles are bound laterally and apically by thin walls which separate the vacuoles from those of the adjacent cells. Scattered over the surface of the episphere between the apical ends of the mucoid cells, are small loculated gland cells (Fig. 37C). These cells contain numerous, small, clear, locules within the cytoplasm. Located basally below these locules are scattered black pigment granules (Fig. 37C).

Mucoid glands are also present on the ventral and lateral surfaces of the pygidium directly below the telotroch (Fig. 37D). These glands are identical to those of the episphere. Lying amongst these cells are small loculated cells very similar to those found in the episphere (Fig. 37D).

Differentiation of the glands of the parathoracic region has begun in the metatrochophore with tentacle buds. The various types of gland cells present at this time are assigned letters that correspond to the fully-developed parathoracic gland cells found in the competent larva. Located on the ventral parathoracic surface in the region of the ventral longitudinal muscles are gland cells which contain purple and blue-staining granules (Fig. 32C). These gland cells will develop into gland cell types A and B of the parathoracic region of the competent larva. Located above these gland cells are other gland cells which contain a pink-staining flocculent material (Fig. 32C). These gland cells will be referred to as type C gland cells. Within the pygidial region, the number of mucoid glands has increased so that below the telotroch there are numerous spherical gland cells containing the reticulate material.

Five distinct types of gland cells, designated A to E, can be found in the parathoracic region of the competent larva based on their morphology and staining properties. The cell types are summarized in Table 2. Located medially surrounding the stomach and intestine are types A and B

(Fig. 38B). Type A is the most conspicuous gland type of the parathoracic region and contains large, pinkish-staining, spherical granules approximately 4 µm in diameter which bear a triradiate mark (Fig. 38B). Gland cells of type B are found amongst cell type A, as well as being located laterally and medially below the parathoracic epithelium. Type B cells are characterized by the presence of blue-staining granules ca. 2 µm in diameter within their cytoplasm (Figs. 33D, 38B). Type C gland cells are found on the ventro-lateral and ventro-medial surfaces of the parathoracic segments as epidermal cells containing either small pink granules about 0.5 to 1 µm in diameter or pink flocculent material (Fig. 38B,C). Type D gland cells are found associated with type C cells on the antero-ventral and antero-medial surfaces of the parathoracic region (Fig. 38C). They contain pinkish-purple granules approximately 1.5 µm in diameter. Type E gland cells are found among the epidermal cells in the antero-ventral parathoracic region and contain blue granules approximately 0.5 µm in diameter (Fig. 38B).

The building organ is now differentiated at this stage as a horseshoe-shaped structure located directly below the mouth on the ventral surface (Fig. 38C,D). The gland cells found in this region are columnar in shape and are types A and B (Fig. 38C,D). There are only 1 to 2 granules per cell. The pygidium contains loculated gland cells amongst the numerous mucoid gland cells (Fig. 38A). The types of gland cells found in the tentacles are described below.

## Tentacles

Rudimentary tentacles first appear in the metatrochophore with tentacle buds, approximately four and one-half weeks after fertilization. These tentacles are very short with a developing ciliary tract located on their ventral surfaces (Fig. 9C). The cells of the ventral surface are cuboidal in shape and are multiciliated. Only one type of gland cell, type D, is present and it contains pinkish-purple granules. These cells are located below the other epidermal cells in the connective tissue of the tentacles. There is no evidence to suggest that pores extend from these gland cells to the surface of the tentacles. Chromatophores containing pigment granules are scattered amongst the epithelial cells on the dorsal surface (Fig. 40A).

Within the center of the tentacles runs a bundle of longitudinal muscle fibers surrounded by connective tissue. At the base of the tentacles, the longitudinal muscle fibers appear to connect to the supraesophageal muscle and to the sides of the esophagus via thin muscle fibers (Fig. 40A).

The tentacles of the competent larva measure approximately 150 µm in length. As described above, the ventral surface has a ciliated tract running along its entire length (Fig. 11A). The cells comprising the ciliary tract are cuboidal in shape and possess many more cilia per cell than the same cells found in the rudimentary tentacles (Fig. 40B). Chromatophores containing pigment granules are scattered among the dorsal epidermal cells. Both type C and E gland cells are found in the epidermis of the tentacles (Fig. 40B). They are found on the dorsal surface and sides of the tentacles. The distal ends of these cells extend into the connective tissue of the tentacles and from their proximal ends extend pores between the other epidermal cells. Unicellular mucoid glands are occasionally found on the dorsal surfaces of the tentacles.

Located on the dorsal surface of the tentacles are presumptive, multiciliated sensory cells (Fig. 40B). These cells usually possess 4 to 6 cilia per cell and their distal ends extend into the connective tissue of the tentacles. Presumably nerve fibers run through the connective tissue, however, they cannot be resolved at the light microscopic level.

Extending the length of the tentacles are longitudinal muscle fibers (Fig. 40C). These muscle fibers form a central core in the distal ends of the tentacles. At the proximal end of the tentacle, there is now a central cavity lined by a thin peritoneum. Within the central cavity lie two distinct coelomic cavities separated by a double peritoneal membrane (Fig. 40D). Following the terminology of Orrhage (1978) for the palps of adult sabellariids, the coelomic cavities will be called the lateral and the medial cavities. The coelomic cavities of the tentacles are separate from the coelomic cavity of the body. Between the peritoneal membrane dividing the lateral and medial cavities lies a blood vessel (Fig. 40D). A ring of muscle surrounds the central cavity and longitudinal muscles insert on its dorsal and ventral coelomic surfaces (Fig. 40D). At the base of the tentacles, the ring muscle connects to the prominent supraesophageal muscle and to the longitudinal trunk muscles situated on either side of the esophagus (Fig. 40A).

## C. SETTLEMENT AND METAMORPHOSIS

The duration of larval development in Sabellaria cementarium ranged anywhere from 6.5 to 8.5 weeks. Larvae displaying the morphological characteristics of competency, however, delayed metamorphosis for over 2 months in the presence of an appropriate metamorphic stimulus. During the process of metamorphosis, the planktonic larva is transformed into a benthic, sedentary juvenile. This chapter will present results from the metamorphic induction experiment and describes the morphological, behavioral, and histological changes occurring in the larvae during metamorphosis.

### Induction of Metamorphosis

Results of the experiment designed to test the effect of different types of substrates on the induction of metamorphosis in the competent larvae are only preliminary. Sufficient numbers of competent larvae were not available to run the experiment on a larger scale. The results, however, are indicative of the observed field distribution.

Table 3 summarizes the results of the experiment. After 18 days, 64% of the larvae in the dish containing the sand from False Bay, San Juan Island had metamorphosed. Sixty percent of the larvae metamorphosed in the dish with the tube sand of S. cementarium, 50% in the dish with the tube sand of Phragmatopoma lapidosa, and 32% in the dish with the tube sand of Indanthrysus ornamentatus. Competent larvae failed to metamorphose in the control dish.

Throughout the summer of 1980 several hundred competent larvae were raised in the laboratory, however, none of the larvae metamorphosed in the culture beakers. This observation is in agreement with the findings in the experimental control. Results of this experiment suggest that the larvae of S. cementarium have a low degree of substrate specificity in the settlement but the presence of sand is essential.

During the course of this experiment, observations were made to determine if the larvae would settle individually or in aggregations. The majority of the larvae were observed to settle in pairs. When this occurred they orientated their tube openings away from each other. There were only two occurrences of what could be interpreted as gregarious settlement. In the dish containing sand of I. ornamentatus 4 larvae settled in close proximity to each other (Fig. 41), while in the dish with the sand from P. lapidosa 5 larvae settled together (Fig. 42).

### Morphology and Behavior of the Metamorphosing Larvae

The metamorphosing larva measures approximately 350 µm in length and is characterized by the anterior rotation of the dorsal tentacles (Fig. 6D). The tentacles are thus parallel with the substrate and the ciliated tracts on their ventral surfaces lead directly into the mouth. The tentacles are very contractile and are capable of extending to lengths equal to that of the body of the larva. The episphere, now called the prostomium (Eckelbarger, 1975) undergoes a reduction in size and its anterior end becomes ellipsoid in shape (Fig. 6D). The eyes have migrated closer together on the dorsal surface of the prostomium and the oral hood fold surrounding the mouth is no longer evident (Fig. 6D). The majority of the prototrochal cilia are lost so that only a few scattered cilia remain on the posterior margin of the prostomium.

As the provisional setae are lost, the settling paleae became evident. The setal sacs originally face laterally, however, they eventually rotate anteriorly so that the paleae come to lie between the tentacles (Fig. 53A,B). At this time the opercular cirri also rotate anteriorly so that they lie on the outside of the paleae. The setal sacs and the opercular cirri are now collectively referred to as the opercular peduncles (Eckelbarger, 1978). There are approximately 6 pairs of settling paleae which eventually will be replaced by the primary juvenile paleae (Fig. 53B). On the ventral surface of the animal, directly below the mouth, the building organ has enlarged to become a prominent horseshoe-shaped structure (Figs. 6D, 53A). The larval pigmentation is still apparent on the dorsal surface of the prostomium. The trunk is divided into 1 thoracic segment, 3 parathoracic segments, 3 abdominal segments, and a pygidium (Figs. 6D, 53A). The segments are delineated on the dorsal surface of the animal by the bands of larval pigmentation.

Due to the reduction in the number of prototrochal cilia the metamorphosing larva is no longer able to swim. It crawls over the substrate employing its tentacles, body segments, and telotroch for locomotion. The pygidium is capable of bending so that the telotroch is raised above the substrate, thus providing the propulsive thrust with its cilia. The ventral ciliation of the tentacles and the undulations of the body also aid in crawling. The direction of movement is determined by the tentacles (Fig. 15C). One of the tentacles extends to its full length while the other is constricted. The larva moves in the direction of the extended tentacle. To change direction, the other tentacle extends and the once extended tentacle becomes retracted.

The larva is constantly testing the substrate during the process of metamorphosis. The larva is capable of using its tentacles to pull sand grains to the building organ and the mouth where they are rubbed against them. The sand particles are then rejected posteriorly by the neurotroch. Often these sand grains are ingested by the larva and their passage through the digestive tract of the larva can be followed.

Following the morphological changes, the larva constructs a mucoid tube. The tube is attached to the substrate and completely surrounds the larva. Initially only the provisional setae are cemented to the tube, but the larva soon begins to cement sand grains to it. The larva uses its tentacles to convey sand grains to the building organ where they are cemented to the tube via the cement from the building organ. Large sand grains are often grasped by the tentacles and pulled directly to the tube. These sand particles are often several times the size of the body of the juvenile and appear to be rubbed against the oral area before they are transferred to the outside of the tube.

The larvae never extend more than one-half of their body lengths outside of their tubes. They rapidly withdraw into their tubes in response to vibrations or shadows and they place a large piece of sand over the tops of the entrance of their tubes which apparently functions as an operculum. The larvae only retract far enough into their tubes so they are covered from the top by the opercular sand grains.

Based upon the observations on several metamorphosing larvae, it appears that the initial events of metamorphosis take approximately 2 days to complete, culminating in the formation of the mucoid tube. Metamorphosis is considered to be complete when the caudal appendage is formed. In S. cementarium this occurs approximately 7 to 10 days after the initial events of metamorphosis.

## Histological Changes During Metamorphosis

To determine the histological changes occurring during metamorphosis, serial sections of juveniles of approximately one, two and three days post-settlement were prepared and they were compared with the sections of the competent larvae. The one day post-settled juvenile displayed the external morphological changes characteristic of metamorphosis, however, a tube had not yet been secreted. The two and three day post-settled juveniles both possessed a mucoid tube, however, only the three day juvenile had begun to cement sand grains to the tube. The structures to be examined are as follows: body wall, muscle system, coelom, alimentary tract, nervous system, and gland cells. Table 4 summarizes the histological changes that occur during metamorphosis.

## Body Wall

The following structures were examined in the body walls of the post-settled juveniles: cuticle, epidermis, and setal sacs.

i) Cuticle:

No changes are observed in the cuticle of the first and second day post-settled juveniles. The cuticle appears to be identical to those of the larval stages. In the two day post-settled juvenile, a thin cuticle can be observed overlying the regions of glandular discharge within the parathoracic region (Fig. 43A). This cuticle is secreted by a thin epithelium lying directly above the longitudinal muscles on the dorsal and ventral surfaces of the animal.

It is not known whether or not the cuticle is secreted before the glands discharged or immediately after they discharged. By three days post-settlement, the cuticle has increased in thickness particularly in the prostomial and parathoracic regions. It appears to be composed of two layers. The outer layer is a thin dark-staining layer and situated below it is a thicker layer which stained lightly. (Fig. 43B). The cuticle is wrinkled in the prostomial and parathoracic regions and has separated away from the underlying epidermal cells (Fig. 47D). Presumably, this is a fixation artifact. The cuticle of the adult appears similar in that it also consists of a darkly-staining layer lying above a lighter staining inner layer (Fig. 52A).

ii) Epidermis:

The epidermis of the first and second day post-settled juveniles is similar to that of the competent larva. The epidermis consists of a simple squamous epithelium, particularly in the regions of the stomach and intestine (Figs. 48A, 51C). The epidermal cells continue to flatten and lengthen while the juvenile becomes thin and vermiform (Fig. 47C). By 2 days post-settlement, scattered, spherical, epidermal mucoid cells are appearing within the prostomium. The mucoid cells contain basally located nuclei and apical spherical vacuoles filled with non-reticulate mucoid substances (Fig. 44D). By 3 days post-settlement these mucoid cells are present along the dorsal, lateral, and ventral surfaces of the prostomial and parathoracic regions

(Figs. 44D, 47C,D). They represent the major cell type found within the epidermis of the anterior region of the juvenile. It is not known if these mucoid cells are retained throughout the life of the juvenile nor is their function known. There are, however, numerous gland cells within the epidermis of the adult (Fig. 52A).

The prototrochal and telotrochal cells are identical to those of the competent larva in the first day of post-settlement except for the reduction in the amount of ciliation (Fig. 44A). During the second day the prototrochal cells are reduced to sparsely ciliated, flattened cells (Fig. 44B), and in the third day the prototrochal cells are no longer distinguishable (Fig. 44D). Presumably they are resorbed rather than shed. The telotrochal cells are gradually decreasing in size but are still recognizable in the three day post-settled juvenile (Fig. 44C).

iii) Setal Sacs:

During the first day of settlement the setal sacs rotate anteriorly, the provisional setae are shed, and the settling paleae become apparent. Histologically, the setal sacs appear similar to those of the competent larva (Fig. 45A). By the second and third days of post-settlement the setal sacs have undergone histolysis (Figs. 45B,C). They have decreased in diameter from 70 µm to about 45 µm. The lateral and basal vacuoles of the setal sacs have disappeared and there has been a reduction in the number of chaetoblast and lateral cells. The chaetoblast cells that secrete the settling and primary paleae are located basally within the sacs, and the lateral cells line the follicles in which the paleae lie (Figs. 45B,C). Histological sections show that the settling paleae are composed of parallel longitudinal channels (Fig. 45A). The setal sacs of the uncinigerous and parapodial lobes did not undergo histological changes at metamorphosis (Fig. 45D). The changes which occurred in the setal sac musculature at metamorphosis will be described in the following section.

## Muscle System

There is considerable reorganization of the muscles within the developing prostomium during metamorphosis while the trunk musculature remains relatively unchanged. In the first day of post-settlement, the setal sac-esophageal muscle complex appears disorientated and is undergoing histolysis. The once prominent supraesophageal muscle remains now as a thin broken band of fibers which is no longer connected to the setal sac muscles (Fig. 46B). The setal sac muscles still form a muscular capsule around the internal surfaces of the setal sacs, however, the anterior and medial connections of the setal sac muscles to the esophagus and to the longitudinal muscles appear as broken bundles of fibers (Fig. 46C). The medial branch of the prototrochal muscle, which once inserted on the esophageal-stomach junction, is no longer distinguishable (Fig. 46A). The setal sacs are still connected to the longitudinal muscles by a thin posterior branch. The longitudinal muscles run along the dorsal and ventral surfaces of the juvenile, extending from the region of the setal sacs to the pygidial region (Fig. 46D). Branches of the dorsal longitudinal muscle project into the parapodial and uncinigerous lobes of the trunk.

There is continued histolysis of the setal sac-esophageal muscle complex and by the third day no trace of the supraesophageal muscle is found (Figs. 47A,D). The setal sac muscles are greatly reduced and they form a thin muscular layer surrounding the internal surfaces of the setal sac in the two day post-settled juvenile (Fig. 47B). By the third day they are barely visible in one micrometer sections (Fig. 47C). There are no traces of the anterior and medial connections of setal sacs to the longitudinal muscles, however, the posterior connection to the longitudinal muscles is retained (Figs. 47B,C). The dorsal and longitudinal muscles give off branches into the regions of the segmental septa which are connected to the muscles of the alimentary tract (Figs. 47C,D). The circular muscles of the body wall are retained as thin bands of muscles between the epidermis and longitudinal muscles.

## Coelom

At metamorphosis a coelomic cavity, the head coelom, is formed within the developing prostomium (Fig. 43C). It appears as a long narrow cavity situated directly anterior to the cerebral ganglion in the antero-dorsal and antero-medial portions of the prostomium. It originates by a separation of the cerebral ganglion from the overlying epidermis. Within the lower portion of the prostomium lies a large coelomic cavity which is the remnant of the primary larval coelomic cavity (Fig. 43C). Located within the trunk are the segmental coelomic cavities. Thin septa which arise from the peritoneal membrane lying against the longitudinal muscles separate the segmental coelomic cavities. Within the pygidium lies a large coelomic cavity which presumably will form the coelom of the caudal appendage of the older juveniles and adults (Fig. 43D).

## Alimentary Tract

The organs of the alimentary tract are retained during metamorphosis, however, the esophagus and stomach undergo modification. During the reorganization of the larval episphere into the prostomium, the antero-ventral glands of the esophagus, which contain a pink flocculent material, are discharged as are the gland cells containing pink-staining granules which are located apically and laterally within the esophageal epithelium (Fig. 48A). Only the gland cells with the purple-staining granules are retained and they are now scarce (Fig. 48A). In the competent larva the esophageal epithelium is composed of 2 cell types, however, by the second and third days of post-settlement only the dark-staining cells are prominent within the esophagus

(Figs 48C,D). The light-staining cells are scarce.

By the second day of post-settlement the vacuolated cells of the stomach have undergone hypertrophy (Fig. 49A). The vacuoles have increased in size several times compared to those of the one day post-settled juvenile (Fig. 48B). Associated with the hypertrophy is a proliferation of the lipid droplets and presumptive zymogen granules within the vacuolated cells and the other cells of the stomach (Fig. 49A). Presumably the lipid droplets provide the nutrition for the juvenile while the stomach is being reorganized. In the three day post-settled juvenile, the vacuolated cells of the stomach obliterate the lumen of the stomach and they have begun to dissociate (Fig. 49C). Phagocytic cells containing numerous presumptive zymogen granules and the cytoplasmic contents of the vacuolated stomach cells are becoming apparent within the stomach epithelium. Within the intestinal lumen are found dissociated vacuolated cells and phagocytic cells (Fig. 49C,D). Also located within the intestine are numerous zymogen granules and cytoplasmic contents of cells which are presumably discharged from the dissociating vacuolated cells. Within the lumen of the esophagus and also in the intestine are found spherical mucoid cells from the epidermal epithelium (Fig. 49D). Presumably they are ingested by the juvenile but how they are released from the epidermis is not known. Vacuolated stomach cells are not apparent within the alimentary tracts of adults so it is assumed that they are completely lost during metamorphosis (Fig. 52B). No morphological changes are noted in the intestine during metamorphosis.

## Nervous System

The juvenile nervous system consists of a cerebral ganglion, circumesophageal commissures, a subesophageal ganglion, ventral ganglia, and paired ventral nerve cords. These ganglia are present in the competent larva and are the major components of the adult nervous system as described by Orrhage (1978).

In the one day post-settled juvenile the bilobed cerebral ganglion has increased in size due to an increase in the number of nerve fibers within the neuropile (Fig. 50A). It occupies a medial position within the prostomium directly below the coelomic cavity. Four prominent optic ganglia are present for the first time and they connect the four eyespots to the cerebral ganglion (Fig. 50A). The eyespots are simple ocelli and resemble those described for Armandia brevis (Hermans and Cloney, 1966; Hermans, 1969) and the trochophores of Harmothoë imbricata (Holborow and Laverack, 1972). The ocelli appear to be of the microvillar (rhabdomeric) type of photoreceptor (Fig. 50A). They are composed of a concave shaped pigment cell which faces antero-dorsally and a microvillar receptor cell lies within the pigment cup. The presumptive microvilli of the receptor cell appear as pale-staining cytoplasmic filaments lying against the granules of the pigment cell. The optic ganglia lead directly into the receptor cells.

The other ganglia have undergone considerable enlargement during metamorphosis and some have shifted in position due to changes within the prostomium. The dorsal and ventral roots of the circumesophageal commissures appear to have shortened and have undergone considerable enlargement as a result of the increase in the number of nerve fibers (Fig. 50B). They have shifted anteriorly and lie close to the ventral epidermis. The ventral ganglia lie directly below the building organ on either side of the esophagus and are connected to each other by a subesophageal ganglion (Fig. 50C). The paired ventral nerve cords arise from the ventral ganglia and extend posteriorly along the ventral surface of the trunk between the muscles and the epithelium (Fig. 50D).

## Gland Cells

The larval glandular elements undergo considerable changes at metamorphosis. In the first day of settlement the apical unicellular mucoid glands are discharged in conjunction with the reorganization of the episphere into the prostomium (Fig. 51A). The lateral mucoid glands of the episphere are still present within the prostomium, however, they have undergone internal reorganization (Fig. 51A). Within the apical vacuoles of these cells, the reticulate material is breaking down into irregular-shaped mucoid droplets. The unicellular mucoid glands are being replaced by unicellular loculated glands which are located on the apical and lateral surfaces of the prostomium (Fig. 51A). These loculated cells are columnar in shape and contain basally located nuclei. Their apical cytoplasm is filled with numerous locules. The mucoid glands of the pygidium are also decreasing in number and are being replaced by the loculated gland cells (Fig. 51B). Associated with the increased size of the building organ, is an increase in the number of gland cells located within it (Fig. 50C). No changes are noted within the parathoracic glands at this time.

By the second day of settlement the mucoid cells of the prostomium are absent and apparently have been replaced by the unicellular loculated glands (Fig. 47B). Within the parathoracic region all five types of gland cells (A-E) are still present, however, there has been a discharge of glandular material (Fig. 43A, 51C). Presumably the parathoracic gland cells are involved in the formation of the primary mucoid tube. On the dorsal and ventral surfaces of the parathoracic region are located large patches of glandular discharge which are marked by the collapse of the epidermal epithelium, so that the epithelium comes to lie over the longitudinal muscles (Fig. 43A). It appears that gland cell types A and B are the major glands discharged within these regions.

In the three day post-settled juvenile there is a reduction in the number of gland cells within the parathoracic region. Type A and type B gland cells are still present but are greatly reduced in number as compared to the competent larva and the one day post-settled juvenile (Figs. 47C, 51D). Gland cell types C, D, and E are present but they are very scarce and are restricted to the apical regions of the parathoracic segments. Within the building organ the gland cells are very abundant despite the fact that sand grains are now being cemented to the mucoid tube (Fig. 51D). Within the pygidium the mucoid cells are almost entirely replaced by the loculated gland cells.

Examination of the adults shows that well-developed gland cells are present within the building organ (Fig. 52D). These glands are involved in the cementing of the sand grains to the tube. Loculated gland cells are not present within the adult prostomium. Within the body segments of the adult the parathoracic glands are no longer present (Fig. 52C). From this observation it can be proposed that the primary function of the parathoracic glands is the secretion of the mucoid tube around the juvenile stages.

## D. DEVELOPMENT OF JUVENILE WORMS

### 5 Days Post-Settlement

The juvenile is approximately 400 µm in length and possesses a single pair of tentacles which appear corrugated (Fig. 53C). A second pair of tentacles is beginning to form posterior to the primary tentacles and they appear as small outgrowths on the lateral surfaces of the body (Fig. 53C). The primary tentacles bear the larval pigmentation on their dorsal surfaces. Located on the ventral surfaces of the primary tentacles are ciliated food grooves, and in the regions of the corrugations are stiff cilia which may be sensory in function (Fig. 53C). There is an aggregation of black pigment granules on the ventral surface of the prostomium (Fig. 53C). The four eyespots have migrated closer together so that they are located medially on the dorsal surface of the prostomium. The opercular cirri have elongated and are located on either side of the primary settling paleae (Fig. 53C). There are two bundles of barbed settling paleae originating from the setal sacs.

The body is divided into 1 thoracic segment, 3 parathoracic segments, 3 abdominal segments, and a pygidium. The larval pigmentation is still apparent on the dorsal surface of the trunk and delineates the segments (Fig. 53C). The uncinigerous lobes have elongated and are facing postero-laterally. The telotroch has disappeared and there is a large reddish-black pigment patch in the region of the pygidium (Fig. 53C). The first trace of the developing caudal appendage is apparent directly posterior to the pygidium (Fig. 53C).

18 Days Post-Settlement

The juvenile measures about 450 µm in length and is characterized by the presence of 3 pairs of tentacles (Fig. 53D, 54A). The primary pair are the longest and originate from the larval stage. Situated at the tips of the primary tentacles are reddish-black pigment spots. A third pair of tentacles is forming anterior to the primary tentacles on either side of the operculum (Fig. 53D). A prominent ciliated food groove runs down the ventral surfaces of all the tentacles (Fig. 54B). Located at the corrugations on the primary and second pair of tentacles are the thick bundles of cilia (Fig. 54B). The sensory cilia that are formed in the larval stages are still apparent on the dorsal surfaces of the primary tentacles.

The prostomium possesses 2 reddish-black eyespots and lacks most of the larval pigmentation which was originally present on the dorsal surface. Nucal spines, while present in most other sabellariid species (Eckelbarger, 1978), are lacking on the dorsal surface of the prostomium in S. cementarium. The opercular cirri situated on either side of the operculum have continued to elongate. There are now three types of paleae present within the setal sacs. Some of the long primary paleae are still present and are located ventrally within the operculum (Figs. 53D, 55B). Primary outer paleae are present and form the opercular cone. Located internally to these outer paleae are the primary inner paleae (Fig. 55B).

Segmentation of the body can be delineated externally and no new segments have formed since the competent larva. Parapodial lobes are now present on the thoracic segments and are directed laterally (Figs. 53D, 54A). Numerous tufts of presumptive sensory cilia are seen on the thoracic segments by SEM examination (Fig. 54C). The abdominal uncinigerous lobes have greatly elongated and face laterally (Figs. 53D, 55A). The bands of larval pigmentation found on the dorsal surface of the trunk are begining to disappear.

Perhaps the most apparent morphological change occurring in the juvenile of this stage is the appearance of the caudal appendage which is achetous and curved anteriorly (Figs. 53D, 55A). At the junction between the abdominal segments and the caudal appendage is located a prominent patch of reddish-black chromatophores which represents the last vestige of the pygidium. The stomach can be detected as two bulbous swellings within the abdominal segments leading into the intestine which extends into the caudal appendage (Fig. 53D).

## 38 Days Post-Settlement

The juvenile measures approximately 550 µm in length and bears three pairs of tentacles (Fig. 56A). The primary pair are still the longest and are distinguished by the reddish-black pigment spots located at their tips. The primary tentacles will form the prostomial palps of the older juveniles and adults. The second and third pairs of tentacles have increased in length but remain much narrower than the primary tentacles (Fig. 56B). The juvenile has lost all of the settling paleae (Fig. 56B). Located within the setal sacs are the outer, middle and inner primary paleae which form the operculum (Fig. 56B).

The larval pigmentation on the dorsal surface of the juvenile has completely disappeared and the larva appears light brown in color (Fig. 56A). The body is divided into 2 thoracic, 3 parathoracic, and 3 abdominal segments (Fig. 56A). The number of setae has increased in the parapodial lobes of both the parathoracic and thoracic segments as have the number of uncini within the uncinigerous lobes. The pigmentation demarcating the junction between the third abdominal segment and the caudal appendage has disappeared. The caudal appendage is approximately one-quarter to one-third the length of the body (Fig. 56A). This is the most advanced stage to which juveniles were raised in the laboratory.

A second pair of setal sacs will form in the later stage juveniles in which more opercular paleae will be formed. The opercular paleae will continue to be replaced until the adult opercular paleae are formed within the sacs. No estimate can be made of the replacement rate or how many sets of juvenile paleae are present in _S. cementarium_. Eckelbarger (1975) estimates the rate of replacement of juvenile paleae in _S. vulgaris_ to be from one to two weeks and presumably the replacement rate for _S. cementarium_ is similar.

# DISCUSSION

## A. LARVAL DEVELOPMENT AND BEHAVIOR

All members of the family Sabellariidae appear to be polytelic in their reproduction. Clark (1978) defines a polytelic species as one that spawns all its gametes in one batch but survives spawning. Polytelic species usually have restricted breeding seasons, however, this study and that of Winesdorfer (1967) have shown that ripe gametes are present in *Sabellaria cementarium* throughout the year. Does *S. cementarium* in fact breed year-round? In light of the assumption by McNulty and Lopez (1969) that the presence of gametes throughout the year means a year-round breeding season, *S. cementarium* would be expected to breed year-round. However, Schroeder and Hermans (1975) point out that this assumption is invalid, as Wilson (1970b) has shown that despite the presence of fertilizable gametes in *S. spinulosa* throughout the year, it only has a 3-4 month breeding season. Furthermore, those species that are known to breed all year are restricted to warm water areas. It, therefore, is unlikely that *S. cementarium* from a cold temperate faunal province, as defined by Briggs (1974), would spawn throughout the year.

It is possible to estimate the season of spawning of *S. cementarium* from the work of Bhaud (1972) who has pointed out that the timing of polychaete reproduction is correlated with latitudinal position; populations from cold waters spawn in the summer, while those from warm waters tend to spawn in the winter. Bhaud (1972) has shown that *S. alveolata* and *S. spinulosa* populations from the Mediterranean spawn in the winter, while those from Scandinavian waters spawn in the summer. Curtis (1978) shows similar patterns for populations of *S. vulgaris* from the Atlantic coast of North America. Based on this information, it is probable that *S. cementarium* has a summer breeding season.

In *S. cementarium*, the gametes are released through the nephridial openings of the gamete bearing abdominal segments which appear to be common to all sabellariids (Waterman, 1934). The sperm of *S. cementarium* has a spherical head, which is defined as primitive by Franzén (1956). This appears to be the common sperm type for the Sabellariidae, however, *Phragmatopoma lapidosa* and *S. floridensis* possess a modified sperm with a long tapered head (Eckelbarger, 1976, 1977). It is interesting that they have such a sperm type and yet have retained external fertilization, as according to Franzén (1956) the modified sperm type is adapted for internal fertilization.

The oocytes of sabellariids range from 75-105 µm in diameter, based on information given in previous studies, and the oocytes of *S. cementarium* fall within this range. The presence of small eggs in the Sabellariidae is correlated with the large numbers of eggs produced and the long term planktotrophic life histories. The cortical reactions and prematuration events in the oocytes of sabellariids were first described by Novikoff (1937) and his findings agree with the other species of sabellariids studied. The time tables of early development of S. cementarium established by Winesdorfer (1967) and Strathmann (1974) agree with the findings of the present study.

Sabellariids are very conservative in their larval development as the larvae of S. cementarium closely resemble those of P. californica (Eckelbarger, 1977), P. lapidosa (Eckelbarger, 1976), S. alveolata (Cazaux, 1964; Wilson, 1929), S. ishikawai (Wu and Ruiping, 1979), S. spinulosa (Wilson, 1929) and S. vulgaris (Eckelbarger, 1975). The only detectable differences are found in the pigmentation patterns of the larvae. These similarities in larval morphology are also paralleled in their adult structures. This similarity between species in both larval morphology and adult morphology appears to be common in polychaetes. For example, in members of the Polydora-complex of the Spionidae (Day and Blake, 1979) and in the Spirorbidae (Potswald, 1965), the similarities in larval morphology are paralleled in their adult morphologies. The larvae of Lygdamis giardi (Wu and Ruiping, 1979) and L. muratus (Wilson, 1977) resemble those of Sabellaria and Phragmatopoma in their early development, however, by the later larval stages they appear to be very different. They possess large oral lip folds which extend around the sides of the episphere, thereby increasing the width of the episphere. Dales (1952) has proposed an evolutionary scheme within the Sabellariidae based upon opercular morphology. According to this scheme, Phragmatopoma and Sabellaria are more closely related to each other than to Lygdamis. This could possibly account for the differences in the larval forms.

A common occurrence in the culturing of sabellariid larvae is the asynchrony in development of the larvae within the same cultures. Wilson (1968b) and Curtis (1973) feel that this may occur in the field between the larvae of the same spat and they suggest it may be an adaptive mechanism to ensure the possibility of successful settlement. As the larvae presumably depend on currents for their dispersal, it would be advantageous to have the larvae stagger their development so that if they encounter a suitable site for settlement, some, at least, would be able to settle.

The SEM studies by Eckelbarger and Chia (1976) and Eckelbarger (1978) on the larvae of P. lapidosa have provided a great deal of information on the external morphologies of the competent larva and juvenile stages, however, the only early developmental stage studied was a young trochophore. The present study, thus, provides the first complete SEM study of larval development in a number of the Sabellariidae. The SEM photographs of S. cementarium are basically a reconfirmation of what has been described at the light microscopic level.

Gland pores were first observed on the surface of the episphere of the 23 hour trochophore. The appearance of the pores corresponds with the internal development of the mucoid cells within the episphere. Gland pores are present on the episphere of all larval stages examined, culminating in the raised gland pores on the episphere of the competent larva. Raised gland pores are also present on the pygidium of the competent larva and recessed gland pores are found on the parathoracic region. The appearance of these pores corresponds to the development of gland cells in the underlying regions of the competent larva. Eckelbarger and Chia (1976) and Eckelbarger (1978) have observed similar gland pores on the competent larvae of P. lapidosa. The presence of the gland pores on the tentacles, however, has not previously been reported.

Eckelbarger (1978) has described the presence of ciliary sensory tufts on the tentacles, pygidium, building organ, dorsal hump, opercular cirri and ventral parathoracic surfaces of the competent larva of P. lapidosa. In S. cementarium, however, sensory tufts are found only on the tentacles, pygidium, and ventral parathoracic surfaces. The sensory tufts of P. lapidosa and S. cementarium resemble those described for the adults of Nereis, Aphrodite (Dorsett, 1976), Polydora (Rice and Simon, 1980) and the flatworm Temnocyphala (Williams, 1978). It is possible that these tufts are either chemoreceptors or mechanoreceptors.

The larvae of S. cementarium initially exhibit a negative geotaxis and become photopositive at 12 days of age. In contrast, the larvae of P. californica (Eckelbarger, 1977), P. lapidosa (Eckelbarger, 1976), and S. vulgaris (Eckelbarger, 1975) are photopositive throughout their entire development, while S. floridensis exhibits no reaction to light throughout the entire larval period (Eckelbarger, 1977). Eckelbarger (1975) has reported that the larvae of S. vulgaris tended to aggregate at the water line in the culture vessels nearest the window illumination as they approached competency, while a smaller number of larvae tended to aggregate away from the light source. As the larvae approached the searching phase they would migrate to the bottom of the culture vessel. The remaining larvae, as they approached competency, would exhibit the same behavior pattern. This has also been reported to occur in Lygdamis muratus (Wilson, 1977) but has not been observed in S. cementarium.

During the searching phase, the larvae of S. cementarium would apply their tentacles and building organ to the sand grains, a behavior which is common to all sabellariids. A behavior not previously reported for sabellariids is the ingestion of sand grains during this period. The larvae of S. cementarium were also observed to apply the tops of their epispheres to the substrate and rotate on their central axis. After a few seconds the larvae would resume swimming. This behavior has only previously been reported for *S. floridensis* (Eckelbarger, 1977). A behavior of the larvae of *L. muratus* (Wilson, 1977) and *S. floridensis* (Eckelbarger, 1977), both of which are species found on soft substrates, is the burrowing through the sand grains. This was also observed in the larvae of *S. cementarium*, however, *S. cementarium* is found on hard substrates. The reason for this behavior is unknown.

Provisional setae are found in all sabellariid larvae and they were first thought to serve a defensive function by Wilson (1929). He stated that small fish would spit out the larvae when they erected their setae. The provisional setae, by increasing the size of the larvae 2 to 3 fold and with the numerous serrations along their lengths, make the larvae difficult to swallow by potential predators. Provisional setae are also found in the larvae of the families Spionidae and Owenidae, where they serve a defensive function (Fauchald, 1974; Schroeder and Hermans, 1975). The spionid and owenid provisional setae resemble those of the sabellariids in that they have annulations along their length bearing numerous tooth-like serrations. The spionids, like the sabellariids, have developed grasping cilia in the telotrochal region to hold the provisional setae to the sides of the body while swimming (Hannerz, 1956; Blake, 1969). The mitraria larva of *Owenia*, however, lacks grasping cilia (Wilson, 1932).

Besides having a defensive function, Wilson (1929) has suggested that the provisional setae play a suspensory role when the larva stops swimming. For S. cementarium this does not appear to be true, as the larvae were never observed to stop swimming and erect their setae unless irritated.

Strathmann et al. (1972) have demonstrated that the trochophore larva of Spirabranchus spinosus is a suspension feeder using an opposed ciliary band system to capture food particles. In this system, one band of cilia, the prototroch, produces the feeding current and the particles are collected downstream in a ciliated feeding groove located between it and a posterior band of cilia, the metatroch. Strathmann et al. (1972) have expressed the opinion that this method of particle capture is common to all polychaete larvae that are suspension feeders. S. cementarium appears to agree with their findings, in that it too is a suspension feeder using an opposed band system. However, as S. cementarium lacks a metatroch the posterior band of prototrochal cilia appears to act as a functional metatroch. The ciliary food groove lies between the two bands comprising the prototroch. This mechanism of particle capture may be common to all polychaete larvae which lack a metatroch. Using the neurotroch as a ciliary rejection mechanism is common to both S. cementarium and S. spinosus, as is the stoppage of feeding when the larva encounters particles too high in concentration.

## B. ORGANOGENESIS AND HISTOGENESIS OF THE PLANKTONIC LARVAE

In those species of polychaetes with planktotrophic larvae, the early trochophores are morphologically incapable of undergoing the metamorphic transformation into the benthic juvenile stages. During the pelagic phase, the trochophore must undergo growth and morphological differentiation in preparation for metamorphosis. During this period of morphological differentiation, the trochophore develops into a metatrochophore and then into a nectochaeta which is the final pelagic stage. The metatrochophore is marked by the segmental arrangement of the ciliary bands and the nectochaeta is a segmented larva in which setae have developed (Schroeder and Hermans, 1975).

We have some knowledge of organogenesis in planktotrophic larval polychaetes from the studies of Åkesson (1961, 1968) on *Pisione remota*, and *Glycera alba*, Segrove (1941) on *Pomatoceros triqueter*, and Wilson (1932) on the mitraria larva of *Owenia fusiformis*. However, except for the study of Åkesson (1961) on *P. remota*, the descriptions of organogenesis are too fragmentary to give a true picture of the sequential histological development of planktotrophic larvae. *P. remota*, like the other members of the errantiate family Pisionidae, has a specialized type of reproduction with internal fertilization (Åkesson, 1961). Therefore, until the present study on *Sabellaria cementarium* there was no detailed account of larval histogenesis and organogenesis in a free spawning species with a typical planktotrophic development. From this study, we now have a better appreciation of the histological transformation of a trochophore into a nectochaeta competent to metamorphose in a species with a typical planktotrophic developmental sequence. As larval development appears to be conservative in the family Sabellariidae, the description of larval organogenesis and histogenesis in *S. cementarium* is felt to be representative of the family.

## Body Wall

### i) Cuticle:

Incorporation of the vitelline membrane into the larval cuticle has been reported for *Sabellaria alveolata* (Wilson, 1929), *S. vulgaris* (Novikoff, 1938), *Phragmatopoma lapidosa* (Eckelbarger and Chia, 1978), *Nereis spp*. (Wilson, 1892), *Nepthys hombergi* (Wilson, 1936b), *Pomatoceros triqueter* (Segrove, 1941), *Pectinaria koreni* (Wilson, 1936b), *Diopatra cuprea* (Allen, 1959) and *Autolytus fasciatus* (Allen, 1964). With the addition of *S. cementarium*, it appears that the incorporation of the vitelline membrane into the larval cuticle is common to all sabellariids.

The presence of the two zones within the cuticle of early sabellariid larvae was first reported by Eckelbarger and Chia (1978) in the larvae of *P. lapidosa*. They were able to trace the two zones of the cuticle to their origin in the vitelline membrane of the oocyte, providing definitive proof that the vitelline membrane is incorporated into the larval cuticle. From the present study it is apparent that the larval cuticle of *S. cementarium* is identical to that of *P. lapidosa*. Both possess cuticles consisting of two zones; Zone I lies above the epidermis and is composed of a loose network of fibrils; and Zone II lies above it and consists of an electron-dense layer of closely packed fibrils through which nonbranching microvilli extend. This cuticle is gradually lost in *S. cementarium* and *P. lapidosa* (Eckelbarger and Chia, 1978) during the trochophore stage and is replaced by an electron-opaque larval cuticle, which is secreted by the underlying epidermis. Associated with this change in cuticle is the appearance of branching microvilli whose tips extend through the surface of the cuticle. Cuticle replacement occurs at a much earlier date in *P. lapidosa* than in *S. cementarium*, 60 hours compared to 5 days. This difference in timing of cuticle replacement can be accounted for by the slower developmental rate of *S. cementarium*, as the morphological stage at which it occurs is identical between the two species.

The larval cuticles of *S. cementarium* and *P. lapidosa* resemble the cuticles of larval *Harmothoë imbricata* (Holborow, 1971; Holborow *et al*., 1969), larval *Arenicola cristata* (Marsden and Lacalli, 1978; Marsden and Pawson, 1981), archiannelids (Brandenburg, 1970;

Rieger and Rieger, 1976), interstitial hesionids (Westheide and Rieger, 1978), and oligochaetes (Potswald, 1971), all of which lack a collagenous grid which is typical of most annelids (Richards, 1978). Within all of these forms, the presence of well-developed microvilli extending through the cuticle is a common feature. Rieger and Rieger (1976) state that such a cuticle is a primitive feature among the Spiralia and they suggest that the function of such a cuticle is to increase the efficiency of uptake of dissolved organic molecules through the epidermis. The uptake of dissolved organic molecules has been documented in oligochaetes (Siebers and Bulhein, 1977) and in archiannelids and interstitial hesionids by Temple and Westheide (1980). It is reasonable to assume that the elaborated microvilli are the site of uptake of the dissolved organic molecules, although there is no direct evidence to support this assumption.

Are the elaborate microvilli of larval polychaetes cuticles, therefore, an adaptation for the uptake of dissolved organic molecules? Chia (1972) has postulated that since epidermal microvilli are common among marine invertebrate larvae, they are probable sites of dissolved organic molecule uptake. Uptake of organic molecules has been demonstrated by Reish and Stephens (1969), who found that the larvae of Neanthes arenaceodentata absorbed labelled glycine and by Bass et al. (1969) who found uptake of lysine by larval Nereis virens was 200 times that of adult worms. The cuticular morphology of these larvae has not been studied, but the adult cuticle of Nereis has been shown to contain microvilli extending through the collagenous grid of the cuticle (Brökelmann and Fischer, 1966; Dorsett and Hyde, 1970). Manahan (1980) has shown that the veliger larvae of Mytilus edulis and Ostrea edulis absorb the greatest concentrations of dissolved organic molecules through the velum, a site which possesses numerous microvilli (Mackie et al., 1976). From this it can be hypothesized that the elaboration of microvilli in the cuticles of S. cementarium and P. lapidosa are adaptations for the uptake of dissolved organic molecules from seawater.

ii) Epidermis:

The epidermis of S. cementarium is a layer of monostratified, columnar epithelium which is of the typical annelid type described by Richards (1978). The structure of the prototrochal cells are consistent with those described for the larvae of Armandia brevis (Hermans, 1966), Harmothoë imbricata (Holborow, 1971; Holborow et al., 1969), P. lapidosa (Eckelbarger, 1978) and Spirobis mörchi (Potswald, 1965).

All are cuboidal in shape and multiciliated, possessing large numbers of mitochondria within their cytoplasm. In S. cementarium, H. imbricata and P. lapidosa the cilia are anchored in the cells by long primary rootlets and by shorter secondary rootlets which lie parallel to the cuticle. This secondary rootlet takes the stress of the cilium and may help to coordinate the metrachronal wave (Richards, 1978). Not all prototrochal cells, however, resemble those described above, as the prototrochal cells of Nereis spp. (Wilson, 1892) and Arenicola cristata (Marsden and Lacalli, 1978) contain large vacuoles within their cytoplasm. Wilson (1892) suggests that these cells may be excretory in function, however, there is no evidence to support this assumption.

iii) Setal sacs:

The setal sacs of S. cementarium are responsible for the formation of the larval setae and for the opercular paleae of the juvenile and adult stages. It must be stressed that the setal sacs are retained after metamorphosis, unlike those found in the larvae of the families Owenidae and Spionidae, which also possess provisional setae. The early ontogeny of the setal sacs in S. cementarium could not be followed, however, they are presumably of ectodermal origin, as all annelid setae are epidermal derivatives (Richards, 1978). Wilson (1932) also assumed that the setal sacs of the mitraria larva of Owenia fusiformis, which bear provisional setae, to be of ectodermal origin.

The setal sacs of S. cementarium first appeared in the 42 hour trochophore as two ectodermally derived cells and throughout their ontogeny the only changes to occur were the increase in the number of chaetoblast and lateral cells and the development of vacuoles. The increase in the cell number resulted in both an increase in the size of the setal sacs and an increase in the number of the provisional setae secreted.

Based on the brief description of the setal sacs in the mitraria of O. fusiformis by Wilson (1932), it appears that they are very similiar in structure to those described in this study of S. cementarium. At the fine structural level, the setal sacs of S. cementarium appear very similiar to the parapodial setal sacs of adult syllids (Bouligand, 1966, 1967) and larval Nereis vexillosa (O'Clair and Cloney, 1974) with regard to the following features: 1) the chaetoblast cells are located at the bases of the follicles and are responsible for setal secretion, 2) the setae lie in follicles bordered by lateral cells, and 3) the distal end of the follicle is lined by the epidermal cuticle. Bouligand (1966, 1967) and O'Clair and Cloney (1974) have demonstrated that the microvilli act as templates on which the setae are deposited. Sections through the setal sacs of S. cementarium also show the presence of microvilli at the bases of the follicles, which may serve as templates in setal formation.

The setae of adult and larval polychaetes contain protein and chitin (Bobin and Mazoué, 1944; Ebling, 1945; Rudall, 1963; Gustus and Cloney, 1973; Richards, 1978) and are composed of parallel longitudinal channels arranged into a central cortex (Bouligand, 1966, 1967; Gustus and Cloney, 1973; O'Clair and Cloney, 1974; Orrhage, 1971; Richards, 1978). It is interesting that the transitory provisional setae of S. cementarium are identical in structure and formation to that of adult and larval setae. From this, it can be concluded that setal structure and formation are conservative ontogenetically within the polychaetes.

### Muscle System

The complexity of the muscle system in the trochophore larva of S. cementarium is unparalleled in the muscle systems previously reported for trochophore larvae. This complexity of the muscle system appears to be related to the defensive behavior of erecting the provisional setae. The setal sac musculature of S. cementarium resembles that of the mitraria larva of Owenia fusiformis (Wilson, 1932), however, Owenia lacks the prototrochal and esophageal muscle connections found in S. cementarium. In Owenia the setal sac muscles are solely responsible for setal erection (Wilson, 1932), while in S. cementarium the esophageal, prototrochal and setal sac muscles act together to erect the setae. The continued hypertrophy of the setal sac-esophageal muscle complex during larval development corresponds to the increasing growth of the setal sacs. The growth of the muscle complex must match that of the setal sacs in order to maintain the defensive behavior. The body wall musculature of the competent larva is of the typical annelid organization.

## Coelom and Segmentation

In the larvae of most polychaetes, the blastocoel forms the primary body cavity. Within the blastocoel are found undifferentiated mesodermal cells which will divide to produce a peritoneal lining, thereby transforming the blastocoel into a true coelom. The differentiation of the mesoderm into a peritoneum occurs after the initiation of segment formation (Anderson, 1959). In the trochophore larvae of S. cementarium, however, the primary body cavity is derived by the splitting of the lateral mesoderm as a blastocoel is lacking. The primary body cavity is thus a true coelom with a peritoneal lining. In the brooded lecithotrophic larvae of Spirorbis mörchi (Potswald, 1965), the primary body cavity also develops as a true coelom. It appears that the development of the primary body cavity as a coelom corresponds to the lack of blastocoelic cavities within these two species. There are, therefore, two methods by which the primary coelomic cavities are formed in larval polychaetes.

In polychaete trochophores, the "M" or mesodermal cells situated at the posterior end of the hyposphere give rise to the ventro-lateral mesodermal bands (Wilson, 1892; Child, 1900; Segrove, 1941; Korn, 1958; Anderson, 1959, 1966, 1973; Åkesson, 1962). In the planktonic trochophores these mesodermal bands are small and inconspicuous (Anderson, 1966, 1973). In S. cementarium, the mesodermal bands agree with Anderson's (1966, 1973) descriptions, in that they consist of small clusters of cells concentrated at the posterior end of the hyposphere. These mesodermal cells form the presumptive prepygidial growth zone, which is involved in segment formation in the later stage larvae.

In larval polychaetes, the development of segmentation is commonly considered to occur by either of two methods. In the Serpulidae (Segrove, 1941), Spirorbidae (Potswald, 1965), Tomopteridae (Åkesson, 1962) and Eunicidae (Åkesson, 1967a), the three anterior segments develop simultaneously prior to metamorphosis and the segmentation of the ectoderm preceeds that of the mesoderm. This method of segment formation has been termed heteronomy by Iwanoff (1928). The characteristics of heteronomy have recently been reviewed by Åkesson (1962) and Schroeder and Hermans (1975). Anderson (1959) and Wilson (1932) have shown that heteronomy is absent in Scoloplos armiger and Owenia fusiformis, respectively. In these two species, the trunk mesoderm exhibits segment formation before the ectoderm is segmentally delineated. This method of segment formation is referred to as homonomy by Schroeder and Hermans (1975). Both heteronomy and homonomy can occur within the same family, as Åkesson (1967a) has shown Eunicids display homonomy, while other members of the family are heteronomous in their segment formation. Åkesson (1967a) believes that when both methods of segment formation occur in the same family, heteronomy is the primitive method and homonomy is derived.

In the Sabellariidae, segment formation is complex and is neither heteronomous or homonomous. In the late trochophore larva the body consists of the episphere and the hyposphere, which contains the setal sacs, prepygidial growth zone and the pygidium. The region of the setal sacs becomes the first thoracic segment. Three segments are gradually formed behind the thoracic segment from the prepygidial growth zone. These are the parathoracic segments and within them, the mesoderm and ectoderm appear to differentiate into segments simultaneously, unlike either heteronomous or homonomous segment formation. Prior to metamorphosis, three more segments, the abdominals, are added from the growth zone. During post-metamorphosis, a second thoracic segment is added between the first thoracic segment and the first parathoracic segment and it does not originate from the prepygidial growth zone. This method of segment formation is unique among the larval polychaetes that have been studied. In agreement with Schroeder and Hermans (1975), I feel that it is related to the formation of the operculum from the first and second body segments. In the adult, the prostomium, first segment, and part of the second segment fuse to form the opercular region.

## Alimentary Tract

In his reviews of polychaete development, Anderson

(1966, 1973) has stated that there are three methods by which gastrulation can occur, depending upon the number, relative size and yolk content of the presumptive midgut cells. In those species with small eggs containing little yolk and numerous small presumptive midgut cells, such as Podarke obscura (Treadwell, 1901) and Hydroides uncinata (Shearer, 1911), gastrulation occurs by invagination. In Arenicola cristata (Child, 1900) and Scoloplos armiger (Anderson, 1959), whose midgut cells are less numerous and situated more ventrally than posteriorly, gastrulation occurs by an inward migration of the presumptive midgut cells, while in those species which have few, large, yolky presumptive midgut cells and no blastocoel, such as Nereis (Wilson, 1892), Tomopteris helgolandica (Åkesson, 1962), and Spirorbis mörchi (Potswald, 1965), the midgut cells are forced internally by the epibolic overgrowth of the surrounding cells. According to Anderson (1966, 1973), S. cementarium being a species with a small egg containing little yolk and small presumptive midgut cells, gastrulation would be by invagination. However, in S. cementarium gastrulation appears to be by epiboly, as there is no blastocoel. It, therefore, appears that the methods of gastrulation are not as closely linked to the nature of the egg and the presumptive midgut cells as previously expressed by Anderson (1966, 1973).

The ontogeny of the alimentary tract in S. cementarium is similar to that previously reported in larval polychaetes (see reviews by Anderson, 1966, 1973). The presence of large vacuolated cells in the upper stomach, as in S. cementarium, have not previously been reported in larval polychaetes. These cells contain numerous vacuoles and bear numerous microvilli on their luminal surfaces. As algal cells have not been identified within the stomach cells of any of the larval stages, it appears that digestion is extracellular. Extracellular digestion is the usual method of digestion in polychaetes (Barnes, 1980; Michel and DeVillez, 1978). Presumably the vacuoles of the upper stomach cells are involved in the production of digestive enzymes, which break down the algal cells. Burke (1978) has shown the existence of similar vacuoles in the stomach cells of echinoplutei, which are responsible for the secretion of enzymes which digest algal cells. In S. cementarium, once the algal cells were broken down the digestive products would be absorbed across the microvilli of the stomach cells. The continued hypertrophy of these cells and the increase in the number of vacuoles within them during larval development, may correlate with the increasing need to digest larger quantities of food during the later stages of larval development.

In the metatrochophore larva, large lipid droplets became apparent within the stomach cells and they were retained through to the competent larva. These lipid droplets may serve as an energy reserve for metamorphosis when the larva would be unable to process food during the histological reorganization of the stomach. Zymogen-like granules also became apparent in the stomach cells of the metatrochophore. They possibly are involved in extracellular digestion of the algal cells and possibly are the precursors of the zymogen producing cells in the adults, as zymogen granules are found within the stomach cells of adult polychaetes where they function in extracellular digestion (Michel and DeVillez, 1978). Gland cells were observed to develop in the esophagus of the larva with tentacle buds and in the competent larva. They are felt to be involved in mucous secretion , as mucous cells are common within the adult esophageal epithelia of both sedentary and errantiate polychaetes (Michel and DeVillez, 1978). It is interesting that gland cells arrive late in the larval development, however, no explanation can be offered for their late development.

## Nervous System

There are numerous studies of the nervous systems in the trochophore and metatrochophore larvae of polychaetes (Korn, 1958, 1960; Åkesson, 1967b; Lacalli and Marsden, 1977; Bubko *et al*., 1979; Ospovat, 1978) at the light microscopic level, but only the studies of Holborow (1971) and Lacalli (in press) provide information on trochophore nervous systems at the fine structural level. From these studies, it is apparent that a cerebral ganglion and prototrochal nerve ring are common to trochophore and metatrochophore larvae and they presumably are responsible for coordination of the swimming and feeding behaviors. In S. cementarium only a cerebral ganglion could be identified in the trochophore larvae and this became apparent at 12 days. Preliminary fine structural observations on the 5 day old larva show the appearance of a prototrochal nerve ring which could not be identified in the light microscope. A cerebral neuropile was not observed with TEM in the 5 day larva. It appears that the prototrochal nerve ring may serve to coordinate swimming and feeding behavior until the appearance of the cerebral ganglion in S. cementarium.

The histological identification of eyespots in the metatrochophore corresponds to the development of a strong, phototactic behavior in this stage. Although optic fibers could not be observed, it appears that the eyes are functional photoreceptors in this and later stage larvae. The eyespots are of the microvillar-pigment cup type described for larval Armandia brevis (Hermans and Cloney, 1966) and for the trochophores of Neanthes succinea (Eakin and Westfall, 1964) and Harmothoë imbricata (Holborow and Laverack, 1972).

By the competent larva, the larval nervous system consists of the same major ganglia as found in the adult sabellariid nervous system (Orrhage, 1978). The presence of a well defined nervous system consisting of a cerebral ganglion, circumesophageal ganglia and a ventral nerve cord has been reported in the fully formed larvae of Armandia brevis (Hermans, 1966), Branchiomma vesiculosum (Wilson, 1936a), Eunice kobiensis (Åkesson, 1967a), Arenicola cristata (Marsden and Lacalli, 1978), Nephtys (Korn, 1960; Bubko et al, 1979), Pectinaria (Korn, 1960), Pisione remota (Åkesson, 1961), Pomatoceros triqueter (Segrove, 1941), Scoloplos armiger (Anderson, 1959), Spirorbis mörchi (Potswald, 1965) and Tomopteris helgolandica (Åkesson, 1962). It is not known if these ganglia are all functional, however, as Marsden and Lacalli (1978) found only a small portion of the ganglionic cells to be functional in A. cristata. It, therefore, appears that the elaborate larval nervous systems of polychaetes are just rudiments of the juvenile nervous systems and they have little function within the larvae. The cerebral ganglion would, thus, be the nervous centre controlling the behavior and metamorphosis of the fully formed larvae.

## Circulatory System

Within the Polychaeta, the major blood vessels of the circulatory system occupy the site of the former blastocoel and the walls of the blood vessels are formed, either from the apposed surfaces of the septa mesenteries, or by the separation of the mesodermal somites from the gut epithelia (Anderson, 1959, 1966). This appears to be true for the development of the circulatory system in the later larval stages of S. cementarium. In S. cementarium the blood vessels occupy the coelom derived from the lateral mesoderm rather than a blastocoelic derivative.

The dorsal and ventral blood sinuses of S. cementarium originate by a separation of the mesoderm from the gut epithelium. The presence of a blood sinus around the gut is a primitive feature within the polychaetes (Anderson, 1959). This method of blood sinus formation has been reported in Owenia fusiformis (Wilson, 1932) and in Arenicola cristata (Lillie, 1905). The formation of the supraesophageal, circumesophageal, dorsal and ventral blood vessels occurs by the separation of the apposed surfaces of the mesenteries. The ventral vessels of Scoloplos armiger (Anderson, 1959), Pomatoceros triqueter (Segrove, 1941), and Spirorbis mörchi (Potswald, 1965) and the dorsal vessel of A. cristata (Lillie, 1905) are also reported to arise in this manner. The blood vessels of S. cementarium are bound by a simple epithelium which is common to all annelids (Hanson, 1949).

From this study it is apparent that the larvae of S. cementarium possess a well defined circulatory system. Similar circulatory systems are also found in the larvae of Armandia brevis (Hermans, 1966), Owenia fusiformis (Wilson, 1932), Scoloplos armiger (Anderson, 1959), and Pomatoceros triqueter (Segrove, 1941). It is not known if they are functional within the larvae, or if they are just structural rudiments of the adult circulatory systems, as blood plasma has not been reported within their vessels.

Acellular blood plasma is found, however, in the larval circulatory system of S. cementarium. Preliminary observations on the adult circulatory system have shown a similar acellular plasma within the blood vessels. Due to the presence of blood plasma within the circulatory system of the larvae of S. cementarium, it is probable that the circulatory system is functional during later larval life.

## Gland Cells

The presence of epidermal gland cells is a common feature among polychaete larvae. In S. cementarium the rudiments of the epispheral unicellular mucoid glands are evident in the 18 hour larva and well developed glands first become apparent in the early trochophore. These unicellular mucoid glands continue to increase in number throughout larval development and also arise in the pygidium. In Phragmatopoma lapidosa, Eckelbarger (1978) has labelled the epispheral and pygidial mucoid glands of the competent larva as type II gland cells and they are identical to those of S. cementarium at the fine structural level. In both species the secretions are composed of irregular fibrillar strands. Mucoid glands have also been identified at the fine structural level in the trochophores of Harmothoë imbricata (Holborow, 1971), however, they differ from those of S. cementarium and P. lapidosa in that the secretions are composed of numerous droplets. Epispheral mucoid glands have also been identified in the trochophores of Glycera alba (Åkesson, 1968) but these are not described at the fine structural level. In S. cementarium, H. imbricata, and G. alba the epispheral mucoid glands do not appear to function within the larva. It is interesting how early in the larval development of these species the mucoid glands appear if they do not function. It is, therefore, assumed that the larval epispheral mucoid glands function during metamorphosis.

In the trochophore larvae of some species of polychaetes the larval glands are known to be involved in feeding. For example, in the trochophores and metatrochophores of Pisione remota (Åkesson, 1961), Lopadorhyncus sp. (Åkesson, 1967b), and Chaetopterus sp. (Werner, 1953), the hypospheral glands secrete mucoid nets which entrap plankton and the larvae then eat the nets with the entrapped food items. The existence of such mucoid nets is unknown in S. cementarium, which is exclusively a ciliary suspension feeder.

It is more common within polychaetes for the epidermal glandular elements to develop within the setigerous larvae. This occurs in Pomatoceros triqueter (Segrove, 1941), Eunice kobiensis (Åkesson, 1967a), Branchiomma vesiculosum (Wilson, 1936a), Spirorbis spp. (Nott, 1973; Quiévreux, 1962; Potswald, 1965, 1978), Owenia fusiformis (Wilson, 1932), Arenicola cristata (Marsden and Lacalli, 1978), and Scoloplos armiger (Anderson, 1959). The larvae of Spirorbis spp., P. triqueter, and B. vesiculosum possess mucoid gland cells which are similar in description to those of S. cementarium, while those of A. cristata and E. kobiensis are highly vacuolated. The descriptions of the mucoid cells in O. fusiformis and S. armiger are too fragmentary to draw comparisons between them and S. cementarium.

The presence of thoracic and parathoracic epidermal gland cells is a common feature among the fully formed larvae of sedentary tubiculous polychaetes. They have been identified in B. vesiculosum (Wilson, 1936a), P. triqueter (Segrove, 1941), Spirorbis spp. (Nott, 1973; Quiévreux, 1962; Potswald, 1965, 1978) and P. lapidosa (Eckelbarger, 1978). In the fully formed larva of S. cementarium there are 5 distinct gland cell types in the parathoracic region, while in P. lapidosa, Eckelbarger (1978) has identified only one cell type, gland cell type I. Cell types A, B, and C in S. cementarium may represent progressive stages of development of a single gland type.

Potswald (1965) described 8 distinct cell types in the thoracic region of the fully formed larva of Spirorbis mörchi, many of which appear identical to those of S. cementarium. In P. triqueter (Segrove, 1941) there are at least three distinct gland cell types in the thoracic region, but they are not described in detail. The presence of different gland cell types within the parathoracic and thoracic regions is a common feature among the larvae of sedentary tubiculous polychaetes. The functional significance of these glands will be discussed in the section on metamorphosis.

## Tentacles

The larval tentacles of sabellariids form the palps of the adult worms, which are located in front of and dorsal to the mouths of the adults. The tentacular or oral filaments do not have their origin during larval development. The larval tentacles are identical histologically to the palps of adult *S. cementarium* (personal observation) and *S. alveolata* (Orrhage, 1978). Orrhage (1978) has shown that the palps of adult sabellariids are homologous in structure to the palps of adult spionids. It, therefore, can be assumed that the larval tentacles of *S. cementarium* are similar in structure to the larval tentacles and adult palps of spionids.

Dales (1952), using Goodrich's (1897) definition of the peristomium of larval polychaetes as the first segment with parapodia and setae, stated that the larval tentacles of sabellariids were of prostomial origin. Hermans (1966) and Schroeder and Hermans (1975) have redefined the peristomium of larval polychaetes as the region including the mouth, prototroch, and metatroch located in front of the most anterior segment which possesses setae. The larval tentacles are thus peristomial in origin. Hannerz (1956) and Blake (1969) have shown the larval tentacles of spionids also to be of peristomial origin, reaffirming the homologies between them and the larval tentacles of sabellariids.

The long ciliary tracts on the ventral surfaces of the tentacles connect to the food groove located between the two ciliary bands of the prototroch. Dales (1952) found that they conveyed food particles into the mouths of larval *Phragmatopoma californica* and thus stated the ciliary tracts were involved in larval feeding. There is no evidence of this in *S. cementarium*, nor has it been noted in any other sabellariid studied. The ciliary tracts appear not to function in the larvae, but in the newly metamorphosed juveniles where they convey sand particles to the mouth from which they are cemented to the tubes. The ciliary tracts are retained throughout adult life (personal observations; Orrhage, 1978) where presumably they serve the same function.

Wilson (1929), in his investigation of the larval development of *S. alveolata*, first referred to the presence of probable sensory tufts on the larval tentacles. Recently, Eckelbarger and Chia (1976) and Eckelbarger (1978) using SEM have revealed the existence of such tufts of stiff sensory cilia on the larval tentacles of *P. lapidosa*. These sensory tufts are identical to those found on the larval tentacles of *S. cementarium*. In one micrometer sections, the tufts appear similar morphologically to those of *P. lapidosa* which Eckelbarger (1978) examined at the fine structural level. He was unable to determine if they were mechanoreceptors or chemoreceptors. As the larval tentacles are definitely involved in substrate examination prior to metamorphosis, it would be interesting to determine, at the fine structural level, if the sensory tufts are, in fact, chemoreceptors. If so, they possibly would be involved in the recognition of the metamorphic inducing chemical which is located in the tube cement (Wilson, 1968b).

## C. SETTLEMENT AND METAMORPHOSIS

Wilson (1958) has emphasized that settlement preceeds metamorphosis in larval polychaetes. This, however, is not a principle common to all polychaetes as Sarvala (1971) has shown that the larvae of Harmothoë sarsi metamorphose in the open water and then settle to the bottom.

From the numerous life history studies of polychaetes (reviewed by Schroeder and Hermans, 1975), we have some appreciation of the external morphological changes at metamorphosis in planktonic larvae. The most apparent changes are the loss of the ciliary bands and provisional setae (if present) and the discharge of glands involved in tube formation. Often there is a reorganization of the prostomial region, as has been reported for arenicolids (Marsden and Pawson, 1981), owenids (Wilson, 1932), sabellariids (Eckelbarger, 1978), sabellids (Wilson, 1936a), serpulids (Segrove, 1941) and spirorbids (Potswald, 1965, 1978).

There is, however, very little information on the histological changes at metamorphosis, particularly in planktotrophic larvae. Various aspects of the histological changes at metamorphosis have been studied in the mitraria larva of Owenia fusiformis (Wilson, 1932) and the endolarva of Polygordius (Woltreck, 1905), both of which undergo a cataclysmic metamorphosis, the lecithotrophic larvae of Branchiomma vesiculosum (Wilson, 1936a) and Spirorbis mörchi

(Potswald, 1965, 1978), and the benthic larva of Arenicola cristata (Marsden and Pawson, 1981). The only studies dealing with planktotrophic larvae are those of Åkesson (1961) on the changes in the nervous system of Pisione remota at metamorphosis and Segrove (1941) on Pomatoceros triqueter, which provides little information. Thus the present study of S. cementarium is the first detailed account of metamorphosis in a species with a typical planktotrophic life history and it is the only study of the histological changes at metamorphosis in a member of the family Sabellariidae.

The developmental rates of sabellariids are very similar, ranging from 2 to 8 weeks (Eckelbarger, 1977). The species studied thus far, however, are either tropical or warm temperate species as defined by Briggs (1974). Thorsen (1950) has stated that the developmental rates of marine invertebrate species living in seas with higher temperatures should be similar to those inhabiting colder, northern seas. S. cementarium is a cold temperate species as defined by Briggs (1974) and its developmental rate of 6.5 - 8.5 weeks falls within the 2-8 week range of other sabellariids. It thus appears that sabellariids agree with the findings of Thorsen (1950) for other marine invertebrates. S. cementarium does, however, have a slower developmental rate than the tropical and warm temperate species due to the colder temperatures at which they are raised, 10-14°C compared to 19-25°C (Eckelbarger, 1975, 1976, 1977). Presumably the greatest differences in developmental rates are due to the differences in culturing techniques. Initially, the larvae of *S. cementarium* were raised using the technique of Eckelbarger (1975) which included aeration of the culture water. This had to be discontinued as it increased the mortality rates of the larvae. This difference in culture technique prevents the direct comparison of the development rates of *S. cementarium* with those of *Phragmatopoma californica* (Eckelbarger, 1977), *P. lapidosa* (Eckelbarger, 1976), *S. floridensis* (Eckelbarger, 1977) and *S. vulgaris* (Eckelbarger, 1975).

It is interesting that the competent larvae will delay metamorphosis even in the presence of an appropriate metamorphic stimulus. This has not been reported before for sabellariid larvae and the reason for this behavior is unknown.

## Induction of Metamorphosis

Based on the results of the settlement experiments, it appears that the larvae of *S. cementarium* exhibit a low degree of substrate specificity in their settlement behavior. Similar findings have been reported for *P. lapidosa*(Eckelbarger, 1976), *S. floridensis* (Eckelbarger, 1977) and *S. vulgaris* (Eckelbarger, 1975) while on the other hand, Wilson (1968a, b, 1970a, b) has shown that the larvae of *S. alveolata* and *S. spinulosa* have high degrees of substrate specificity. To account for these differences, Eckelbarger (1977) has proposed that substrate specificity may be a method of reproductive isolation among sympatrically occurring species. S. alveolata and S. spinulosa occur sympatrically (Wilson, 1929) and the differences in substrate specificity may result in geographically isolated populations. Reproductive isolation would, therefore, arise by habitat isolation. P. lapidosa, S. floridensis and S. vulgaris lack a high degree of substrate specificity in their settlement behavior and each is geographically isolated from other sabellariid species. Therefore, they would not be expected to develop substrate specificity in their settlement to preserve reproductive isolation. This explanation cannot be applied to S. cementarium, as it occurs sympatrically with another sabellariid, Idanthyrsus ornamentatus. They are often found within the same aggregations and preliminary observations have shown that they both contain ripe gametes at the same time and cross fertilization will occur between the two species. Perhaps reproductive isolation in the field occurs either by a temporal separation in spawnings or by hybrid inviability.

Adults of the family Sabellariidae occur mainly in small aggregations or in reefs and with the exceptions of Lygdamis muratus (Wilson, 1977) and S. floridensis (Eckelbarger, 1977), which are solitary species, the larvae show gregarious settlement behavior. Crisp (1979) has proposed two methods by which reaggregation can occur in marine invertebrate larvae; the larvae can settle in response to contact with their own species, A, or in response to another species, B. Wilson (1968a, b, 1970 a, b) has demonstrated that reaggregation in sabellariids occurs by the former method. The larvae of *S. alveolata* and *S. spinulosa* are attracted to the cement of adult tubes, tube fragments, and the tubes of newly metamorphosed juveniles. Within the tube cement is an insoluble, metamorphosis inducing factor with which the larva must make contact before the metamorphic response is elicited (Wilson 1968a, b, 1970a, b). The factor is presumably similar to the insoluble, tanned, proteinaceous materials which elicit the settling response in *Balanus balanoides* (Knight-Jones, 1953) and *Crassostrea virginica* (Crisp, 1967).

*S. cementarium* is found in small aggregations in Griffin Bay, Washington. As there were only two instances of gregarious settlement in the laboratory one would not expect gregarious settlement in the field. How, therefore, do the lab results relate to the field distribution? Eckelbarger (1975) has found that *S. vulgaris* juveniles initially build tubes in a solitary manner, spatially separated by at least 1 cm. As more larvae settled, they filled in between the settled larvae forming small aggregations. The tubes grew together and, thus, form aggregations similar to those in the field. Straughtan (1972), Sentz-Braconnet (1968) and Klöckner (1976) have described similar behavior in serpulid larvae. It thus appears that a similar situation occurs in *S. cementarium* but due to the small numbers of larvae used in the settlement experiment, this behavior could not be detected. Why species like S. cementarium, S. vulgaris and P. lapidosa show a low degree of substrate specificity and yet exhibit gregarious settlement behavior is unknown.

## Morphology and Behavior of the Metamorphosing Larvae

The morphological changes observed during metamorphosis in S. cementarium agree with those described for P. lapidosa (Eckelbarger, 1976, 1978), P. californica (Eckelbarger, 1977), S. alveolata (Cazaux, 1964; Wilson 1929), S. spinulosa (Wilson, 1929), S. vulgaris (Eckelbarger, 1975) and Lygdamis muratus (Wilson, 1977). Metamorphosis, thus, follows a characteristic pattern in the Sabellariidae. The first observable changes are the shrinkage of the episphere and the loss of the prototroch and oral hood. The tentacles then rotate anteriorly and the provisional setae are lost. The setal sacs containing the settling paleae and the opercular cirri also rotate anteriorly. The body of the larva elongates, the building organ swells and the telotroch is lost. These morphological changes are then followed by the construction of the tube.

A behavior pattern not previously reported in sabellariid larvae during metamorphosis is the testing of the substrate by using the tentacles to pull sand grains to the mouth. Once at the mouth the sand grains are either ingested or they are rejected posteriorly by the neurotrochal cilia. The only other larva in which the ingestion of sand grains has been observed is the metamorphosing pelagosphera larva of sipunculids (M. Rice, personal communication), where this behavior is felt to be a method of testing the substrate. Another behavior not previously reported, is the determination of the direction of movement during the crawling phase by the larval tentacles.

The method of tube construction in *S. cementarium* agrees with that described for *S. vulgaris* (Eckelbarger, 1975), *S. alveolata* (Wilson, 1929) and *L. muratus* (Wilson, 1977). The juveniles initially form mucoid tubes which are attached to the substrate. They then seize sand grains in the tentacles where they are conveyed to the mouth along the ciliary tracts of the tentacles. These sand grains are then cemented to the tube by the secretions from the building organ. In *S. cementarium* the provisional setae are cemented into the tube, a behavior which has not previously been reported for sabellariids. Eckelbarger (1975) and Rees (1976) have shown that the newly metamorphosed juveniles of *S. vulgaris* select smaller sand grains over larger ones during the initial events of tube building. Juvenile *S. cementarium* were not observed to have a grain size preference during tube building. A behavior, apparently unique to *S. cementarium*, is the placement of a large sand grain over the top of the entrance to the tube, which serves as an operculum, as the settling paleae provide little protection to the juveniles from potential predators.

The settling paleae of S. vulgaris and P. lapidosa serve as a tool for the shaping and arranging of the sand grains during tube formation and for scraping fouling material from the opening (Eckelbarger, 1975), although this was not observed in S. cementarium. The settling paleae of S. cementarium resemble those described for S. vulgaris (Eckelbarger, 1975). Settling paleae are reported from all sabellariids except S. floridensis (Eckelbarger, 1977). The settling paleae are not homologous to the paleae of the adult operculum and they will be replaced by the primary paleae in the older juveniles. Nucal spines which are reported for S. vulgaris (Eckelbarger, 1975), S. alveolata (Wilson, 1929) and S. spinulosa (Wilson, 1929), are absent in S. cementarium.

## Histological Changes During Metamorphosis

### Body Wall

i) Cuticle:

The cuticle of the first and second day post-settled juveniles resembles that described for the larval stages of S. cementarium and P. lapidosa (Eckelbarger and Chia, 1976; Eckelbarger, 1978). However, by the third day of settlement the juvenile cuticle is composed of two layers: a thick, lightly-staining layer situated above the epidermis and a thin dark-staining layer on top of it. This resembles the cuticular morphology described by Eckelbarger (1978) at the fine structural level for a juvenile P. lapidosa two weeks after settlement. At the light microscopic level, the cuticle of the adult S. cementarium appears to be similar to that of the three day post-settled juvenile. It thus appears that the adult cuticle of sabellariids is formed during metamorphosis.

ii) Epidermis:

During metamorphosis, the epidermis maintains its monostratified appearance which is characteristic of the epidermis in both the larvae and adults of S. cementarium. The transformation of the cells from columnar to squamous is associated with the remodeling of the larval cells into the vermiform shape of the juvenile stages. The presence of mucoid cells is a common feature of the epidermis of polychaetes (Daly, 1973; Dorsett and Hyde, 1970; Storch and Welsch, 1972; Westheide and Rieger, 1978) to which numerous functions have been ascribed (reviewed by Richards, 1978). Mucoid glands are found developing in the epidermis of the lower prostomium, thoracic and parathoracic segments of the two and three day post-settled juveniles. They presumably are the rudiments of the adult epidermal mucoid glands.

One event common to the metamorphosis of larval polychaetes is the loss of the prototroch, which has been described as occurring by one of several methods. The cilia may drop off and the prototrochal cells undergo atrophy as in *Armandia brevis* (Hermans, 1966), the prototrochal cells may be cast off as in *Spirorbis mörchi* (Potswald, 1965, 1978), or the entire prototroch and associated cells of the episphere may separate away from the epidermis as in *Owenia fusiformis* (Wilson, 1932) and *Branchiomma vesiculosum* (Wilson, 1936a). In *S. cementarium* it occurs by the first method. Most of the cilia drop off during the initial events of metamorphosis, however, some remain until the second day. The prototrochal cells undergo atrophy, so that by the third day they are no longer distinguishable. An exception to this phenomenon is *Arenicola cristata* (Marsden and Pawson, 1981) which does not lose its prototrochal cells at metamorphosis. The significance of this situation is unknown (Marsden and Pawson, 1981).

iii) Setal Sacs:

Wilson (1932) has shown that the setal sac of the mitraria larva of *Owenia fusiformis* lose their provisional setae and undergo histolysis at metamorphosis. This is what would be expected, as the setal sacs are larval structures specifically adapted for the formation of the provisional setae, which serve a defensive function for the larva (Wilson, 1932). However, in sabellariids the setal sacs are retained at metamorphosis and they form the juvenile and adult opercular paleae. Hartman (1944) suggests that the setal sacs and their corresponding segment are precocious in their development as they serve the adult function of paleae formation. If the setal sacs are in fact adult structures as suggested by Hartman (1944), they would not be expected to undergo histological change at metamorphosis. This study of S. cementarium, however, shows they do.

As stated earlier, the setal sacs rotate anteriorly and lose their provisional setae during the initial events of metamorphosis. During the first day the sacs are histologically identical to those of the competent larva, however, by the second and third days of post-settlement they have decreased in size. This reduction in size is due to a loss of both chaetoblast and lateral cells, probably by histolysis. It can be argued that the setal sacs are no more precocious in their development than any other of the larval structures which will form adult tissues, as they underwent histological change at metamorphosis. The setal sacs are larval structures that precociously form the settling paleae in the larvae, but are not adult structures within the larvae.

## Muscle System

The histolysis of the setal sac-esophageal muscle complex at metamorphosis is associated with the loss of the defensive function of the setal sacs. Since the setal sacs remain stationary in the adult, the elaborate muscle system associated with them is no longer required and it, therefore, undergoes histolysis. A thin band of muscle is retained around the coelomic surface of the setal sacs in the juvenile and adult stages (Ebling, 1945). This is the only remnant of the setal sac-esophageal muscle complex. Accompanying the loss of the prototrochal cells at metamorphosis, is the histolysis of the prototrochal muscles. The loss of the prototrochal muscles has also been reported in *Arenicola cristata*, although unexpectedly, as the prototrochal cells are retained (Marsden and Pawson, 1981).

### Coelom and Segmentation

During metamorphosis in *S. cementarium*, the cerebral ganglion separates from the overlying epidermis to form a coelomic cavity, the head coelom, which is separate from the primary coelom. A similar separation of the cerebral ganglion from the overlying epidermis has been described in *Armandia brevis* (Hermans, 1966, 1978), *Arenicola cristata* (Marsden and Pawson, 1981), *Branchiomma vesiculosum* (Wilson, 1936a), *Pisione remota* (Åkesson, 1961), and *Scoloplos armiger* (Anderson, 1959), although it is not clear if the cavities formed are true coeloms as in *S. cementarium*.

There is no reorganization of the segmental coelomic cavities of the trunk at metamorphosis. The body of the adult sabellariid is divided into the opercular region composed of the prostomium and first segment, 2 thoracic segments, 3 parathoracic segments, the abdominal segments and a caudal appendage (Fauchald, 1977). After segment formation in the larva, which is described in the preceding chapter, the larval trunk consists of 2 thoracic, 3 parathoracic, and 3 abdominal segments except for S. cementarium in which there is only one thoracic segment. Although the second thoracic segment will form during the juvenile of S. cementarium, it appears that the segmentation of the adult is established prior to metamorphosis. The growth zone in the juvenile responsible for the formation of the additional segments would be located in front of the caudal appendage, while in S. cementarium there would be an additional growth zone between the first thoracic and first parathoracic segments, responsible for the second thoracic segment. This zone, however, cannot be detected in one micrometer sections of the early juveniles.

## Alimentary Tract

The histological reorganization of the larval alimentary tract during metamorphosis is a common phenomena in the planktotrophic larvae of marine invertebrates. For example, in the veliger larvae of nudibranch molluscs (Bickell, 1978; Bickell et al., 1981) and in echinoderm larvae (Burke, 1978; Cameron, 1975; Chia and Burke, 1978), the larval guts undergo a drastic reorganization into the adult guts. Until the present study on S. cementarium, histological reorganization of the larval stomach within polychaete larvae has only been reported in Owenia fusiformis (Wilson, 1932) and Polygordius (Woltreck, 1905). Both of these polychaetes, however, possess highly modified larvae and undergo an atypical metamorphosis. Thus, this study provides the first evidence for the histological reorganization of the larval stomach in a polychaete with a typical planktotrophic life history.

In S. cementarium, the larval stomodeum and esophagus were found to form the adult esophagus which has also been reported to occur in Pomatoceros triqueter (Segrove, 1941) and Owenia fusiformis (Wilson, 1932). In contrast, the larval esophagus and pharynx of aphroditids, pisionids, nepthyids, phyllodocids, and spionids produce a pair of envaginations which appear to function as imaginal discs for the replacement of the larval pharngeal epithelium at metamorphosis (Anderson, 1973) and in Scoloplos armiger the larval stomodeum forms the adult pharynx (Anderson, 1959).

The glands within the esophageal epithelium are discharged during the first day of metamorphosis. As stated earlier, it is not known if these glands are functional within the late larval stages. It is possible that they develop only to function during metamorphosis as they may provide an enzymatic secretion which is involved in the digestion of the oral hood and other larval tissues of the episphere. The loss of glands from the esophagus has not previously been noted in larval polychaetes.

During the metamorphosis of larval polychaetes, the larval stomach and intestine usually form the adult midgut epithelium (Anderson, 1973). However, in S. cementarium it appears that the larval stomach must undergo histological reorganization before it forms the adult midgut, while the larval intestine is directly transformed into the adult intestine. This reorganization occurs in the region of the vacuolated cells of the upper stomach. During the first day of metamorphosis, the vacuolated cells undergo a drastic increase in size so that they obliterate the esophageal-stomach junction. By the third day of metamorphosis, these cells have dissociated, have begun to slough off and phagocytic activity is apparent within this region. The larval stomach cells will be replaced by the stomach epithelium. As stated earlier, there is a proliferation of cells with zymogen-like granules in the later stage larvae. This was originally related to the extracellular digestion of the algal cells but it may also be related to the digestion of the larval stomach cells which accumulate in the lower stomach and intestine during this reorganization period.

A build-up of lipid droplets occurred in the stomach cells of the late stage larvae and it may provide an alternative source of nutrition during metamorphosis, as it is doubtful if the juvenile would be able to digest food during this reorganization period. Support for this assumption comes from Bickell (1978) who found a build up of lipid in the digestive glands of the veliger larvae of Doridella steinbergae, a nudibranch mollusc, prior to metamorphosis. She felt that the lipid may serve as an energy resource for metamorphosis, as the larval gut undergoes a drastic reorganization at metamorphosis.

The histological reorganization of the stomach at metamorphosis is presumably related to the differences in the diets between the larvae and adults of S. cementarium. Although both the larvae and adults are suspension feeders, the larvae feed exclusively on small phytoplankton while the adults feed on larger planktonic organisms (Fauchald and Jumars, 1979). It appears that the stomach of larval sabellariids is a specialized larval structure specifically adapted to the diet of the larvae. It must undergo histological reorganization into the adult stomach while the other portions of the alimentary tract form the corresponding structures in the adult. This, however, appears to be common to other polychaetes, as in Owenia and Polygordius, the stomach is the only portion of the alimentary tract which undergoes reorganization at metamorphosis.

## Nervous System

Although the separation of the cerebral ganglion from the overlying epidermis is common to all polychaetes, an increase in the size of the cerebral ganglion has not previously been reported. In fact, the cerebral ganglion of Pomatoceros triqueter undergoes a decrease in size (Segrove, 1941). Marsden and Pawson (1981) found a burst in neuron and synaptic differentiation in the cerebral ganglion of *Arenicola cristata* at metamorphosis, however, the ganglion did not increase in size. The rapid increase in the size of the cerebral ganglion of *S. cementarium* may be due to a similar burst in neuron differentiation. As pointed out by Marsden and Pawson (1981) the rapid differentiation of neurons at metamorphosis may be a common phenomenon among marine invertebrate larvae as a similar event occurs within the abdominal ganglia of the veliger larvae of the opisthobranch mollusc *Aplysia californica* at metamorphosis (Schacter *et al*., 1979a, b). Presumably this increase in neuron and synaptic differentiation is related to the transition of the larval nervous system to that of the adult.

An interesting occurrence at metamorphosis is the differentiation of the optic ganglia and the increase in the differentiation of the eyes, however, adult sabellariids lack prostomial eyes. To undergo such a differentiation the eyes must, therefore, serve a function in the juvenile stages of *S. cementarium*. One possible function is the detection of shadows which may serve as a predator avoidance mechanism. Juvenile worms will extend up to one-half their body length out of their tubes to collect sand grains for tube building. Preliminary observations show that the larvae will retract into their tubes in response to a shadow over the entrance of the tube. As the adults do not extend their bodies out of the tube, they would no longer require such a predator avoidance mechanism and the eyes would degenerate. Additional evidence to support this assumption comes from the fact that the eyes appear externally to have disappeared by the 38 day post-settled juvenile, at which time the juvenile no longer extends out of the tube.

### Gland Cells

The discharge of gland cells is common among the metamorphosing larvae of sedentary polychaetes. In *S. cementarium*, the epispheral and pygidial mucoid glands are discharged in the initial events of metamorphosis and they are presumably involved in attachment. The discharge of the epispheral glands is felt to be responsible for the shrinking of the episphere. Discharge of epispheral glands is also associated with the reorganization of the episphere at metamorphosis in *Arenicola cristata* (Marsden and Pawson, 1981), *Branchiomma vesiculosum* (Wilson, 1936a), *Owenia fusiformis* (Wilson, 1932), *Pomatoceros triqueter* (Segrove, 1941), and *Spirorbis mörchi* (Potswald, 1965, 1978).

The parathoracic glands of *S. cementarium* are responsible for the secretion of the primary tube. Presumably all five gland cell types are involved in tube formation, with some providing the mucoid materials and others providing the catalyzing substances. The discharge of the parathoracic and thoracic glands also occurs in *Arenicola* (Marsden and Pawson, 1981), *Owenia* (Wilson, 1932), Pomatoceros (Segrove, 1941), Spirorbis (Potswald, 1965, 1978) and Branchiomma (Wilson, 1936a), where they are involved in tube formation.

## D. DEVELOPMENT OF JUVENILE WORMS

The juvenile stages of *S. cementarium* resemble the juveniles of *Phragmatopoma californica* (Eckelbarger, 1977), *P. lapidosa* (Eckelbarger, 1976), *S. alveolata* (Cazaux, 1964) and *S. vulgaris* (Eckelbarger, 1975). The rates of growth for the juveniles of *S. cementarium* are much slower than for these warm temperate and tropical species. The differences are presumably due to the differences in the water temperatures at which the juveniles were raised.

Eckelbarger (1977) states that the primary paleae of *S. floridensis*, *S. spinulosa*, and *S. vulgaris* resemble the outer opercular paleae of the juveniles and adults and that they are probably homologous. However, in *S. cementarium* the primary paleae resemble the primary opercular paleae of the juveniles but not those of the adults. This is because the adults of *S. cementarium* possess an atypical outer opercular paleae when compared to other members of the genus *Sabellaria*.

An interesting feature of the juveniles of *S. cementarium*, is the presence of ciliary sensory tufts on the dorsal surfaces of the opercular and thoracic regions. This has not previously been reported for sabellariid juveniles. These ciliary sensory tufts resemble those described by Dorsett (1976) for adult *Nereis* and *Aphrodite aculeata*, which are believed to be mechanoreceptors. It is possible that the sensory tufts of *S. cementarium* juveniles are also mechanoreceptors. Preliminary observations have shown that the juveniles will retract into their tubes in response to vibrations in the water. The ciliary tufts presumably are responsible for the perception of the vibrations causing the juveniles to retract into their tubes.

# TABLES AND FIGURES

Table 1: Chronology of Larval Development and Metamorphosis of Sabellaria cementarium in the Laboratory (10 - 14°C).

| Developmental Events | Time (post-fertilization) |
|---|---|
| 1st polar body | 1.5 hours |
| 2nd polar body | 2 hours |
| 1st polar lobe and 1st cleavage | 2.5 hours |
| 2nd polar lobe and 2nd cleavage | 4 hours |
| 3rd polar lobe and 3rd cleavage | 5.5 hours |
| Free swimming pretrochophore; negatively geotactic | 18 hours |
| Early trochophore with apical tuft and prototroch | 23 hours |
| Trochophore with provisional setae; appearance of defensive behavior | 60-65 hours |
| Feeding trochophore | 3.5-4 days |
| Metatrochophore; positively phototactic | 18 days |
| Formation of tentacle buds; positively phototactic | 4 weeks |
| Nectochaeta competent to metamorphose; positively phototactic; positively geotactic | 5-6 weeks |
| Larvae begin crawling behavior; metamorphosis; provisional setae lost; anterior rotation of tentacles; building of tubes | 6-8 weeks |
| Juvenile; appearance of 2 pairs of tentacles; settling paleae and larval pigmentation still present; formation of caudal appendage | 7-9 weeks |
| Juvenile; appearance of 3rd pair of tentacles; outer and inner paleae present in operculum; few settling paleae still present; caudal appendage well | 10-12 weeks |

Table 1: Continued

| | |
|---|---|
| formed; loss of larval pigmentation | |
| Juvenile; complete loss of settling paleae; inner, middle, and outer primary paleae present; appearance of 2nd thoracic segment | 12-15 weeks |

Table 2: Summary of the Gland Cell Types in the Parathoracic Region of the Competent Larva.

| Gland | Staining Property (Richardson's Stain) | Location | Granule Morphology |
|---|---|---|---|
| A. | pink | building organ, entire parathoracic region | granules numerous, *ca*. 4 µm, triradiate mark |
| B. | blue | building organ, medial and lateral parathoracic region | granules numerous, *ca*. 2 µm |
| C. | pink | ventro-lateral and ventro-medial parathoracic surfaces | granules scarce, *ca*. 2 µm, may have flocculent material |
| D. | pinkish-purple | antero-ventral and antero-medial parathoracic surfaces | granules scarce, *ca*. 0.5-1 µm |
| E. | blue | antero-ventral parathoracic surfaces | granules scarce, *ca*. 0.5 µm |

Table 3: Induction of Metamorphosis in *Sabellaria cementarium*

| Substrate Provided | Number of Competent Larvae | Number Metamorphosed After 18 Days | % Metamorphosis |
|---|---|---|---|
| False Bay, San Juan Island sand | 25 | 16 | 64 |
| *Sabellaria cementarium* tube sand | 25 | 15 | 60 |
| *Phragmatopoma lapidosa* tube sand | 30 | 15 | 50 |
| *Idanthrysus ornamentatus* tube sand | 25 | 8 | 32 |
| Control (no sand) | 25 | 0 | 0 |

Table 4: Summary of the Morphological Changes at Metamorphosis in *Sabellaria cementarium*

| Tissue, Cell Types and Other Structures | Competent Larva | 3 Days Post-settlement |
|---|---|---|
| Epidermal gland cells | | |
| A (parathoracic, building organ) | yes | yes; reduced in number |
| B (parathoracic, building organ) | yes | yes; reduced in number |
| C (parathoracic) | yes | scarce |
| D (parathoracic, tentacles) | yes | scarce |
| E (parathoracic, tentacles) | yes | scarce |
| mucoid (episphere, pygidium) | yes | no |
| loculated (episphere, pygidium) | scarce | many |
| Cuticle | single layer | bilayer |
| Epidermis | monostratified; columnar | monostratified; squamous; appearance of mucoid cells |
| Coelomic cavities | | |
| primary | yes | yes |
| segmental | yes | yes |
| head | no | yes |
| Setal Sacs | large; provisional setae | reduction in size; loss of provisional setae |

Table 4: Continued

| | | |
|---|---|---|
| Nervous System | | |
| cerebral ganglion | yes | yes; increase in size |
| circumesophageal commissures | yes | yes; shortening |
| subesophageal ganglia | yes | yes |
| optic ganglia | no | yes |
| paired ventral nerve cords | yes | yes |
| Muscle System | | |
| setal sac-esophageal muscle complex | yes | no; histolysis |
| circular, longitudinal trunk muscles | yes | yes |
| Alimentary Tract | | |
| esophageal gland cells | yes | no |
| vacuolated cells of stomach | small | hypertrophy and dissociation |
| Prototroch | yes | no; atrophy |
| Telotroch | yes | yes |
| Neurotroch | yes | yes |
| Oral hood | yes | no |
| Tentacles | pointing posterior | pointing anterior |

Figure 1: Diagram of the anterior end of *Sabellaria*
(after Ebling, 1945)

Legend:

B - Branchia
BO - Building organ
DS - Dorsal setae
I - Inner opercular paleae
O - Outer opercular paleae
TF - Tentacular filaments
U - Uncinigerous lobe
VS - Ventral setae

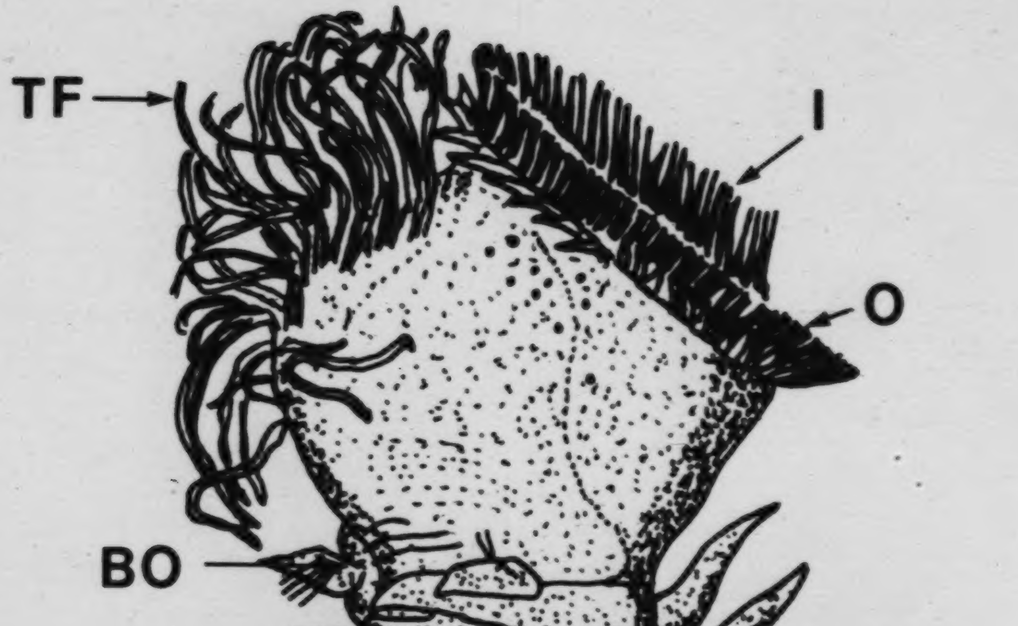

TF
I
O
BO
B
VS
DS
U

Figure 2: Scanning electron micrographs (SEM) of the anterior end of adult Sabellaria cementarium.

A. Right lateral view of the prostomium of an adult worm showing the prominent operculum characteristic of sabellariids. Scale bar = 0.5 mm

B. Frontal view of the operculum of an adult worm. Note the numerous tentacular filaments on either side of the operculum. Scale bar = 0.5 mm

Legend

OC - Opercular cirri
OP - Operculum
T - Tentacles

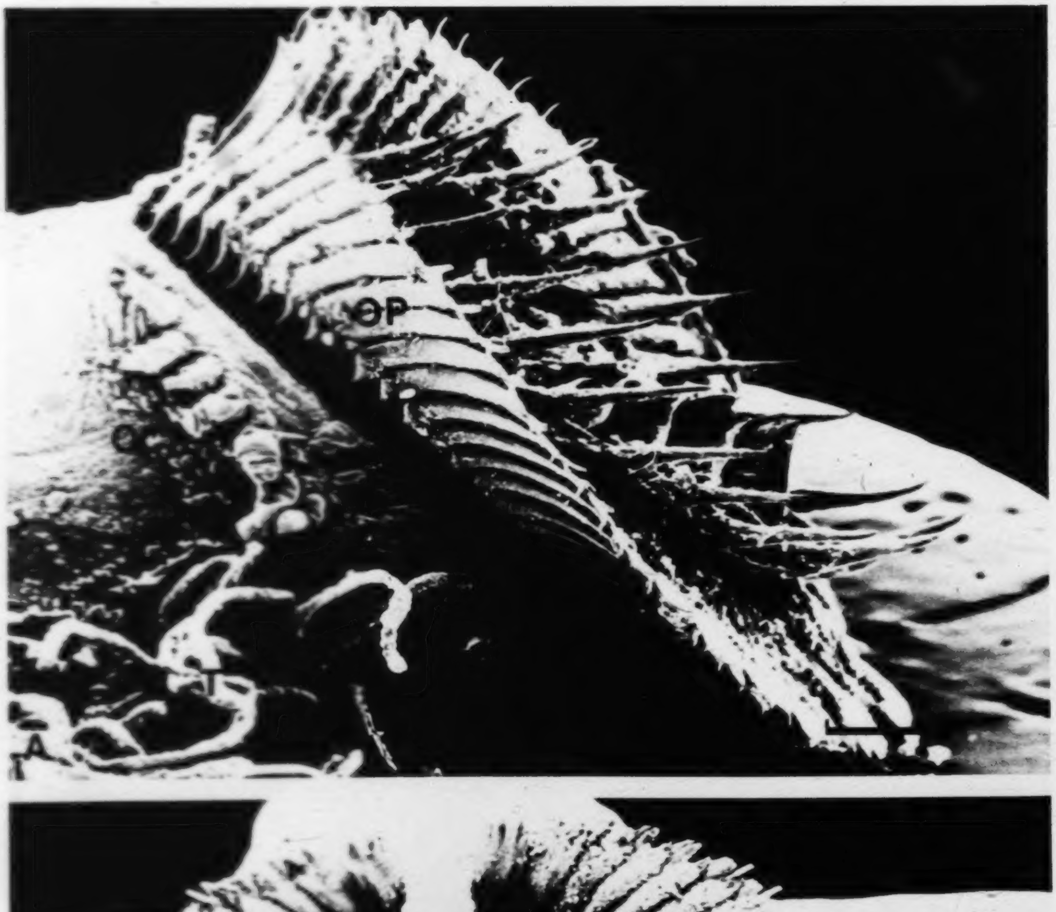

OP
O
T
A
B

Figure 3: Early embryogenesis of *S. cementarium*.

A. Unfertilized oocytes after germinal vesicle breakdown. Note the ellipsoid shape of the vitelline membrane. Scale bar = 30 µm

B. Fertilized oocyte which has released first and second polar bodies. Note the rounding up of the vitelline membrane. Scale bar = 30 µm

C. Oocyte in trefoil stage. Note the large first polar lobe. Scale bar = 30 µm

D. Late cleavage stage. Scale bar = 30 µm

Legend:

PB - Polar body
PL - Polar lobe
VM - Vitelline membrane

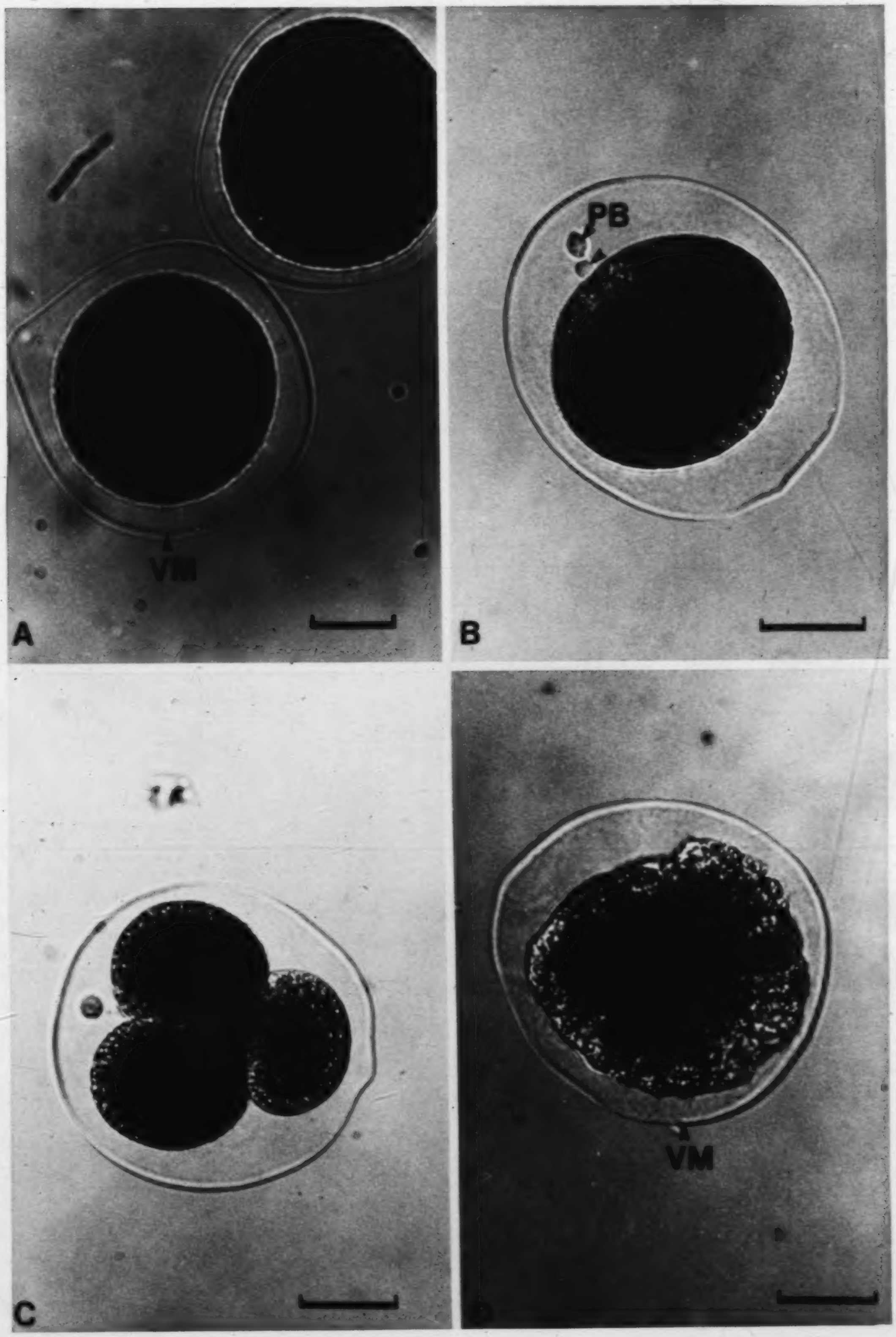

VM
A
PB
B
C
VM
D

Figure 4: Development of the planktonic larvae of *S. cementarium*, I.

A. Free swimming, 18 hour blastula. Note the prominent vitelline membrane surrounding the blastula. Scale bar = 40 µm

B. Trochophore 23 hours old with long apical tuft. Scale bar = 40 µm

C. Trochophore 44 - 46 hours old. Moderate squash of the larva accounts for its increase in size. Scale bar = 40 µm

D. Trochophore 62 hours old with newly formed provisional setae. Note the faint outline of the developing gut. Scale bar = 40 µm

Legend:

AT - Apical tuft
PS - Provisional setae
PT - Prototroch
VM - Vitelline membrane

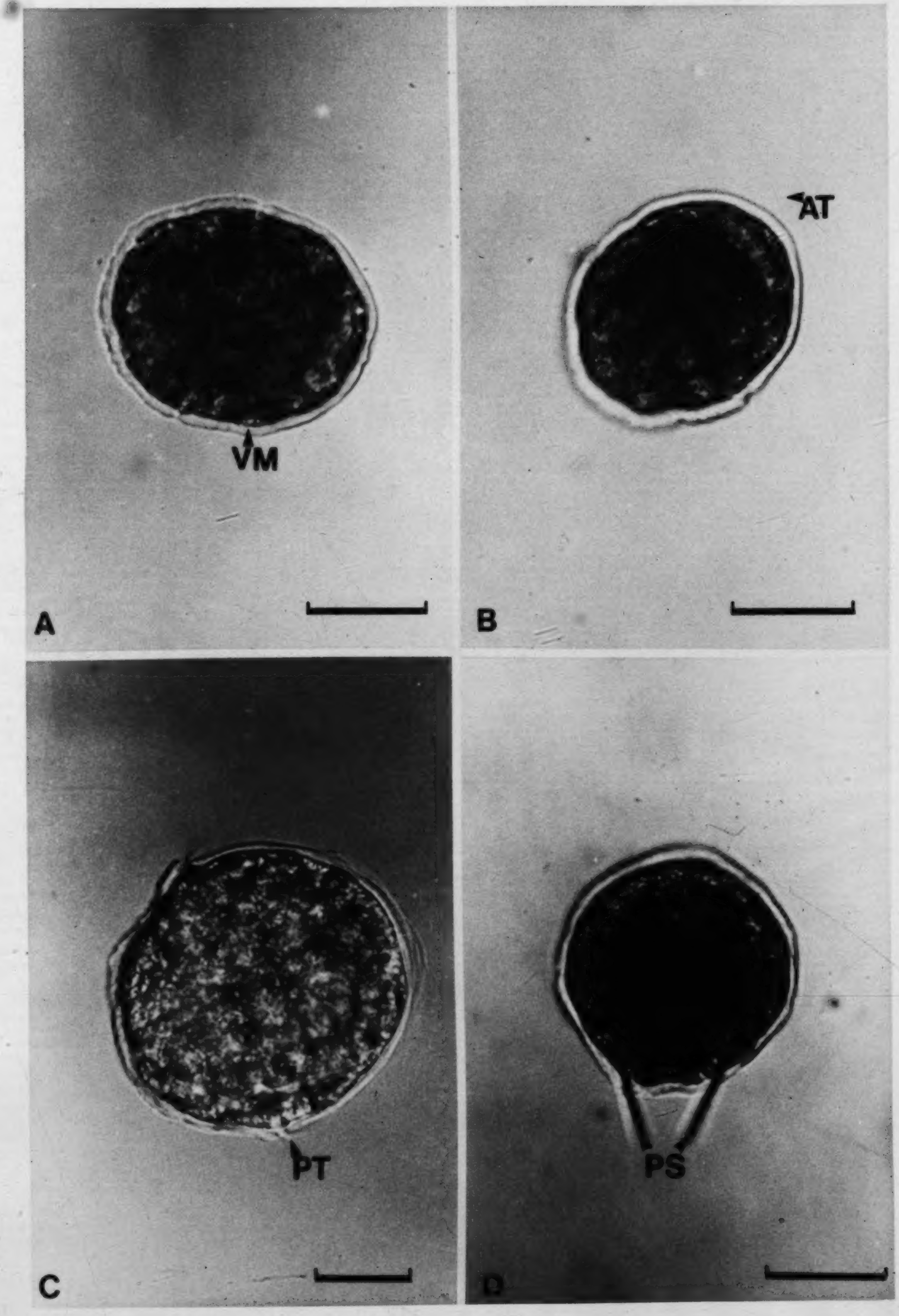

VM
A
AT
B
PT
C
PS
D

Figure 5: Development of the planktonic larvae of _S. cementarium_, II.

A. Dorsal view of 65 hour trochophore. Note the presence of two pairs of provisional setae and the forming gut. Scale bar = 40 µm

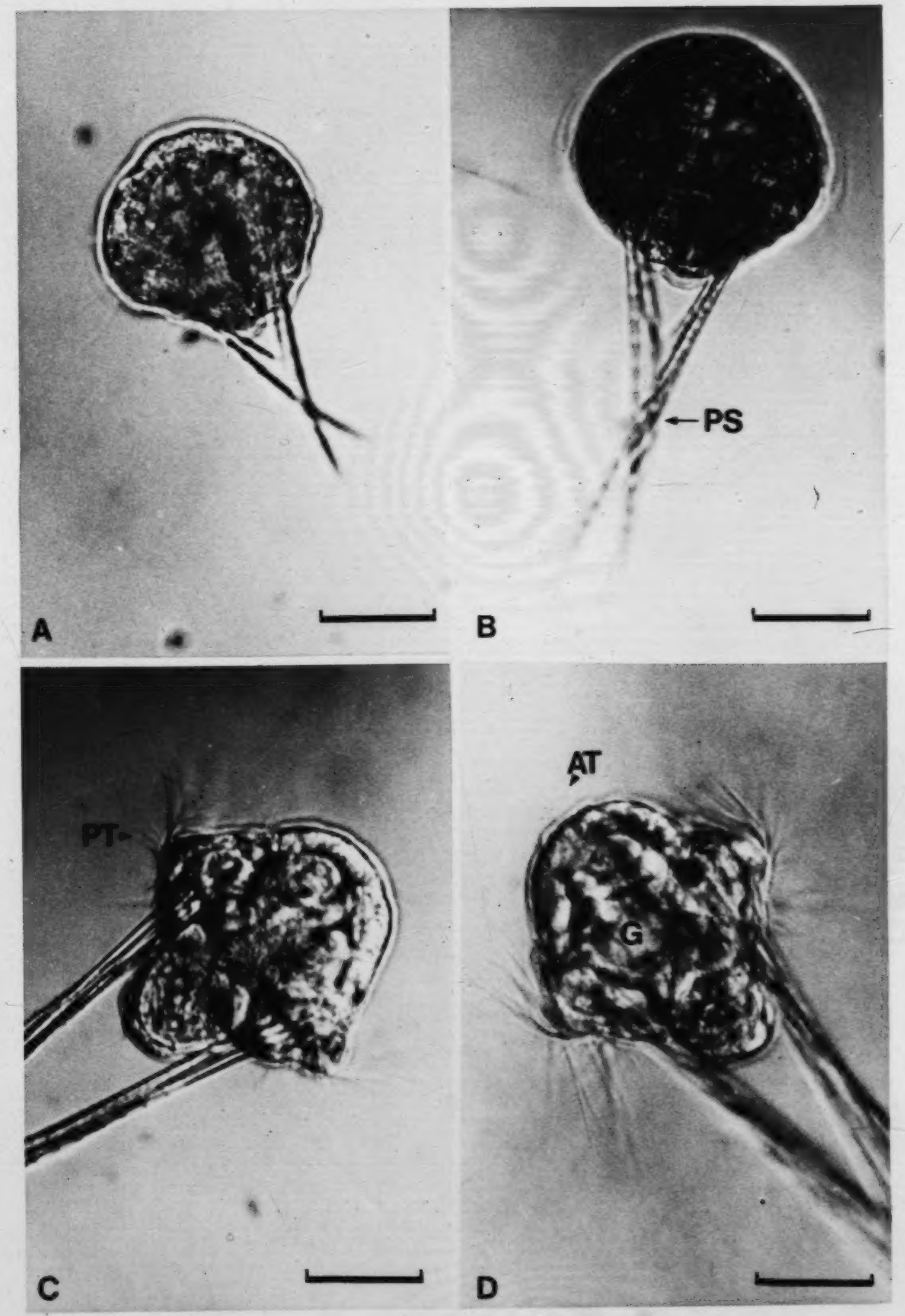
B. Dorsal view of 3.5 day old trochophore. Note the length of the provisional setae compared to the body of the larva. Scale bar = 40 µm

C. Dorsal view of 5 day old trochophore showing the developing alimentary tract. Scale bar = 40 µm

D. Dorsal view of 12 day old trochophore. The eyespot can be seen on the right side. Note the well-defined alimentary tract. Scale bar = 40 µm

Legend:

AT - Apical tuft
E - Eyespot
G - Gut
PT - Prototroch



A
B
PS
C
PT
D
AT
G

Figure 6: Development of the planktonic larvae of S. cementarium, III.

A. Dorsal view of 18 day old metatrochophore. Scale bar = 40 µm

B. Right, lateral view of a larva developing tentacle buds approximately 4.5 weeks old. Note the prominent oral hood fold. Scale bar = 40 µm

C. Left, lateral view of a competent larva, 7 weeks old. Scale bar = 40 µm

D. Ventral view of a metamorphosing larva in which the tentacles, opercular cirri, and setal sacs have begun to rotate anteriorly. Arrows indicate the decrease in size of the prostomium. Also note the enlarged building organ and provisional setae which have begun to drop out. Scale bar = 40 µm

Legend:

BO - Building organ
E - Eye
M - Mouth
OC - Opercular cirri
OH - Oral hood fold
PS - Provisional setae
ST - Stomach
T - Tentacle
TB - Tentacle bud
UL - Uncinigerous lobe

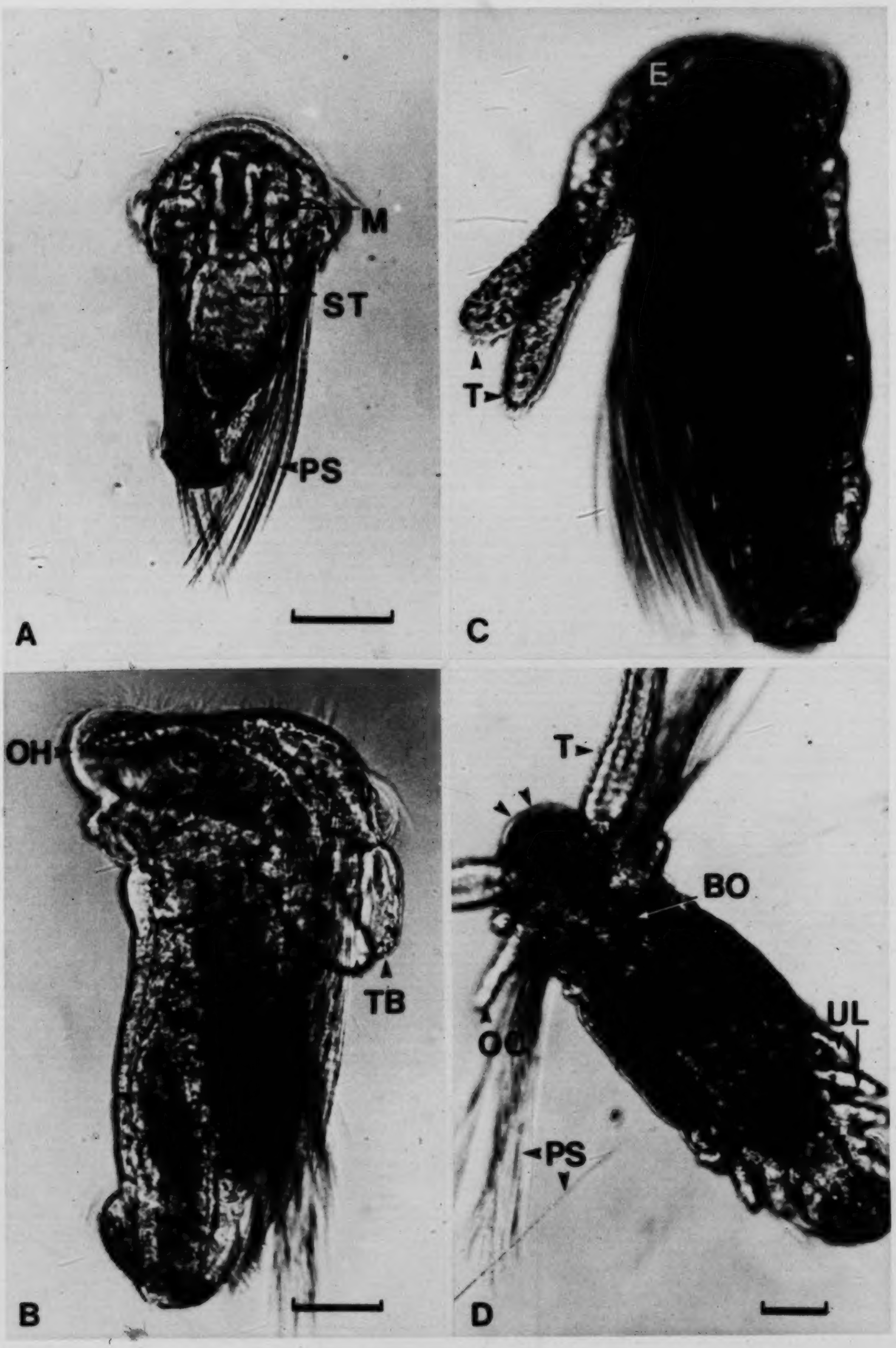

M
ST
PS
A
E
T
C
OH
TB
B
T
BO
UL
OC
PS
D

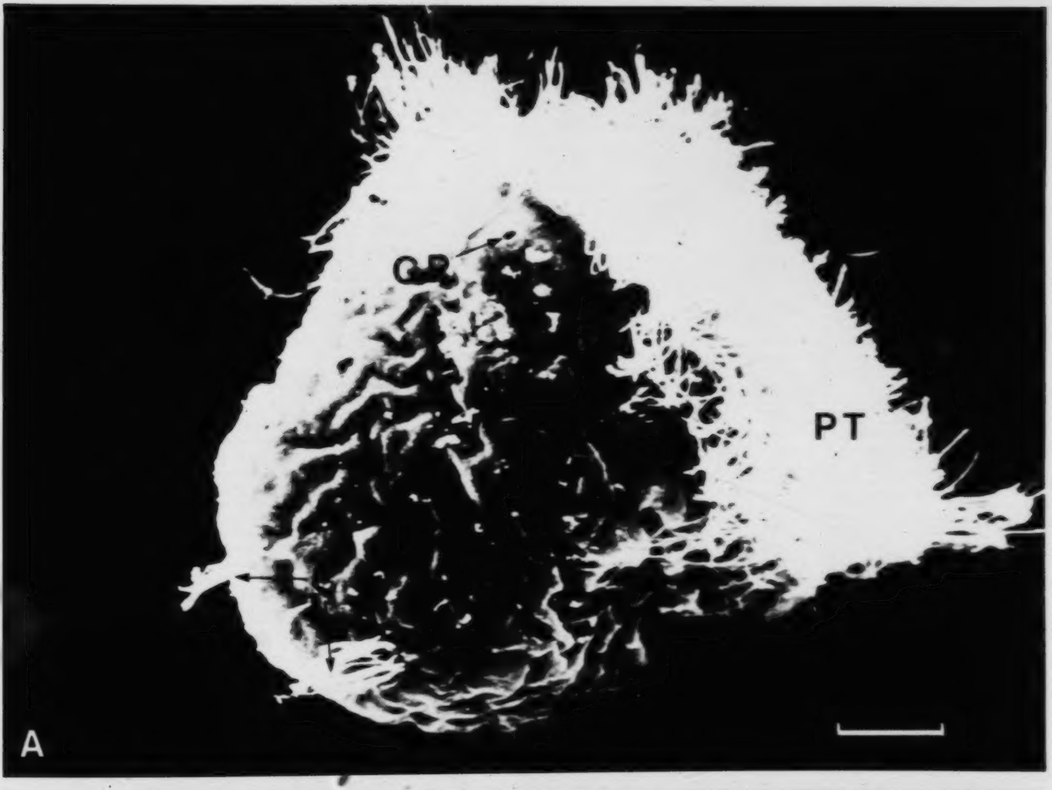
Figure 7: SEM of 23 hour trochophore and 65 hour trochophore.

A. Dorsal view of a 23 hour trochophore. The apical end is obscured from view. Note the gland pore below the prototroch and the posterior cilia on the hyposphere. Scale bar = 10 µm

B. Lateral view of a 65 hour trochophore with 2 pairs of provisional setae. Note the long cilia of the apical tuft and the gland pore above the prototroch. Scale bar = 20 µm

Legend:

AT - Apical tuft
C - Cilia
GP - Gland pore
PS - Provisional setae
PT - Prototroch


GP
PT
A

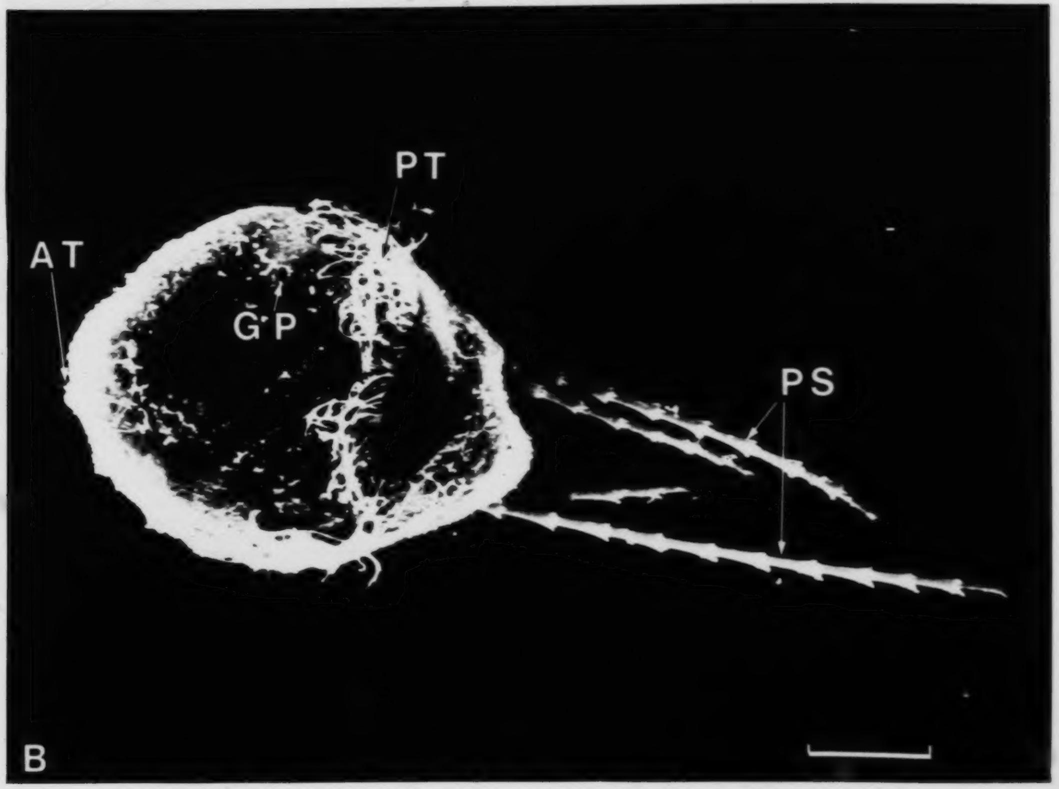

PT
AT
GP
PS
B

Figure 8: SEM of 5 day old trochophore.

A. Ventral view of a larva showing the prominent oral ciliation and the neurotroch leading from the mouth. Note the prototroch is composed of 2 bands of cilia. Scale bar = 10 µm

B. Apical tuft of the same larva. Scale bar = 10 µm

C. Dorsal view of a larva showing the length of the provisional setae in relation to the body and the gap in the prototroch on the dorsal surface. Scale bar = 20 µm

Legend:

AT - Apical tuft
M - Mouth
NT - Neurotroch
PS - Provisional setae

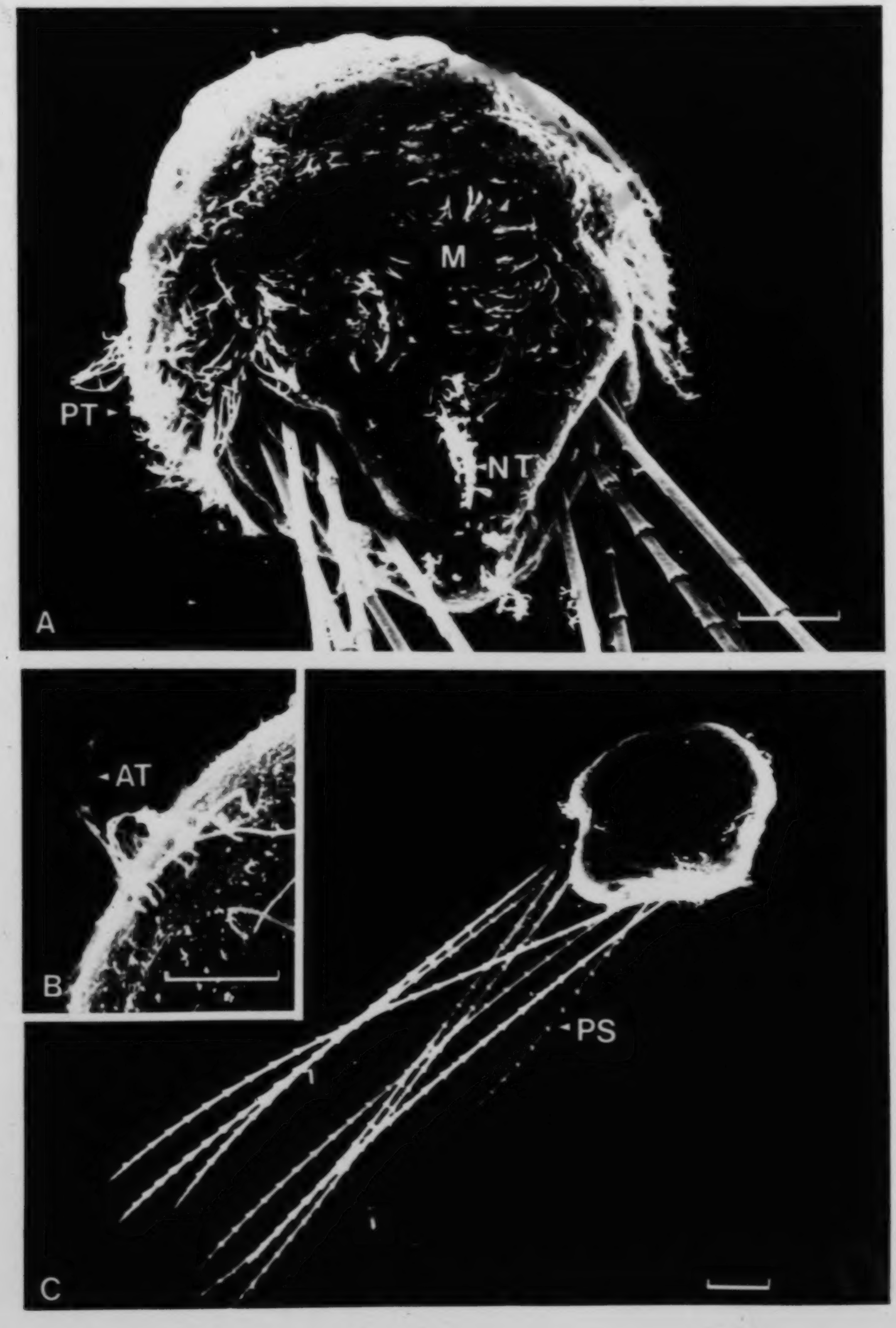

M
PT
NT
A
AT
B
PS
C

Figure 9: SEM of metatrochophore larva and larva with tentacle buds.

A. Left, lateral view of metatrochophore showing the oral hood fold surrounding the mouth on the ventral surface and the prominent telotroch and grasping cilia on the pygidium. Scale bar = 20 µm

B. Lateral view of the grasping cilia from the pygidium of the metatrochophore larva. Note how they are composed of individual cilia. Scale bar = 10 µm

C. Dorsal view of larva with developing tentacle buds. Note the gap in the prototroch on the dorsal surface of the episphere. Scale bar = 50 µm

Legend:

GC - Grasping cilia
OH - Oral hood fold
PT - Prototroch
TB - Tentacle bud
TT - Teletroch

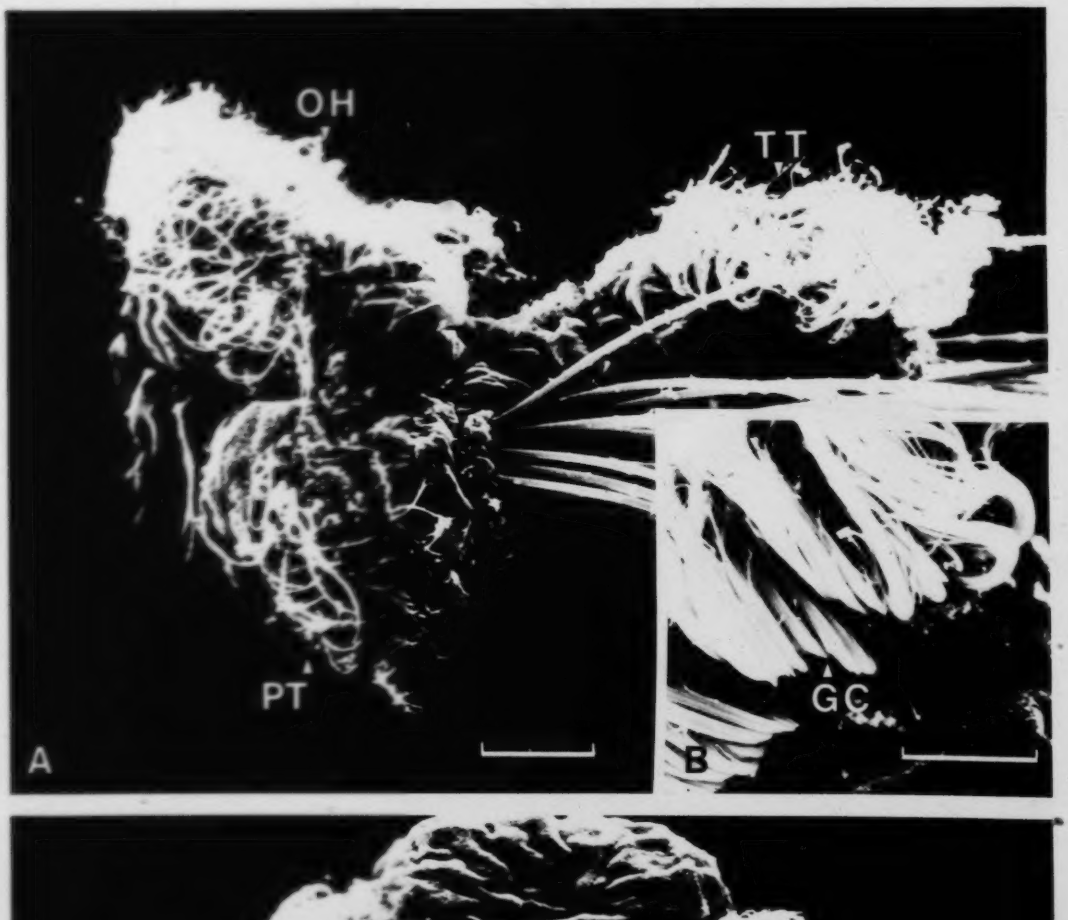

OH
TT
PT
GC
A
B
PT
TB
C

Figure 10: SEM of competent larvae.

A. Left, lateral view of a competent larva. Note that the prototroch is composed of two ciliary bands. Scale bar = 50 µm

B. Ventral view of a competent larva showing the neurotroch which extends from the mouth to the telotroch. Scale bar = 100 µm

C. Dorsal view of a competent larva. Note the dorsal hump situated between the tentacles, the parapodial lobes on the parathoracic segments, and the uncinigerous lobes on the abdominal segments. Scale bar = 50 µm

Legend:

AS - Abdominal segments
E - Episphere
DH - Dorsal hump
NT - Neurotroch
P - Pygidium
PS - Parathoracic segments
PT - Prototroch
T - Tentacle
TS - Thoracic segment
TT - Telotroch

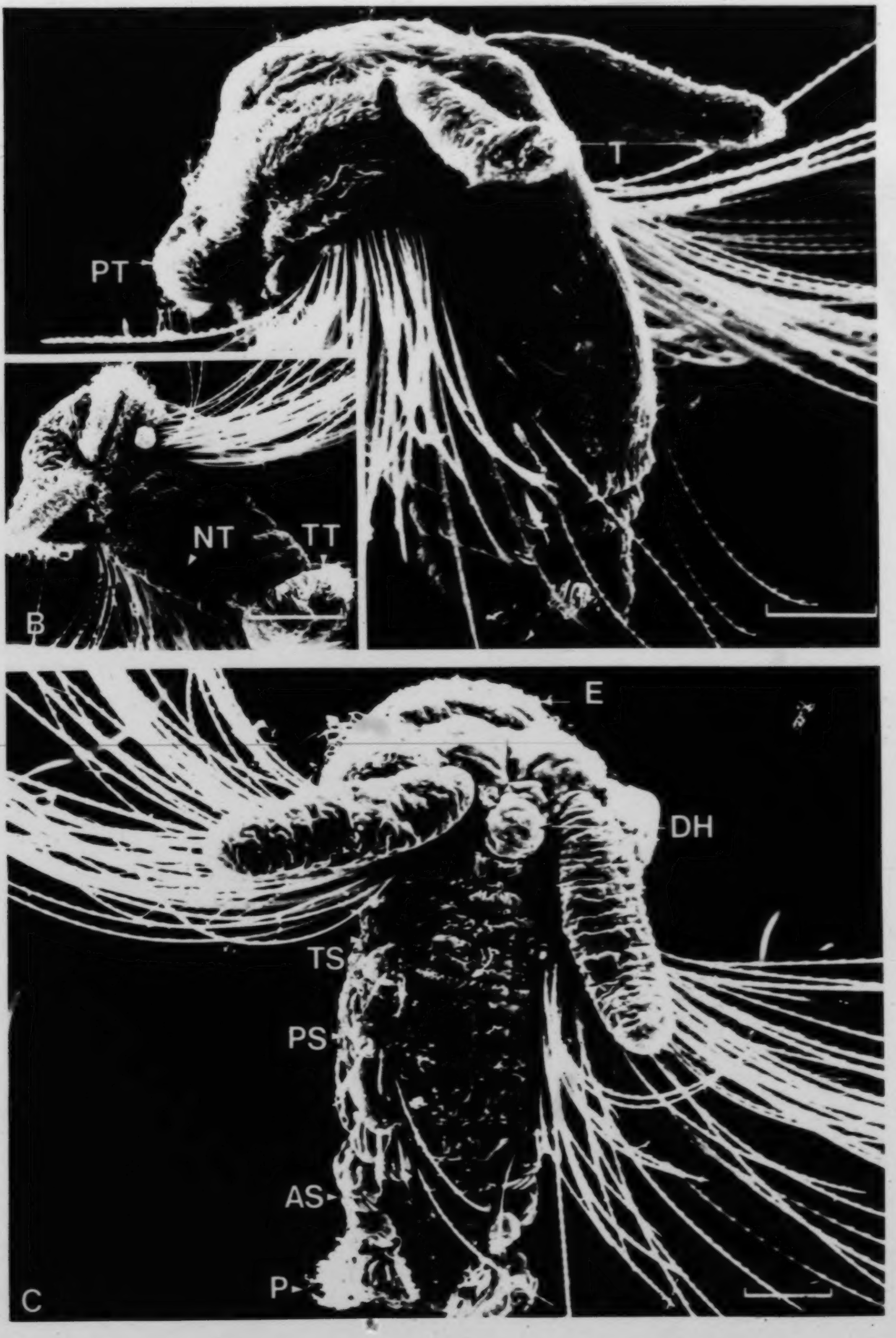

T
PT
NT
TT
B
E
DH
TS
PS
AS
P
C

Figure 11: SEM of a competent larva showing the tentacle and mouth.

A. Lateral view of the tentacle showing the ciliated groove which extends along the entire length of the ventral surface. Scale bar = 10 µm

B. Dorsal view of the tentacle showing the ciliary sensory tufts. Scale bar = 4 µm

C. Ventral view of the episphere showing the mouth surrounded on either side by the lip folds. Note also the building organ located below the mouth and the opercular cirri. Scale bar = 20 µm

Legend:

BO - Building organ
CT - Ciliary tract
LF - Lip fold
M - Mouth
OP - Opercular cirri
ST - Sensory tuft

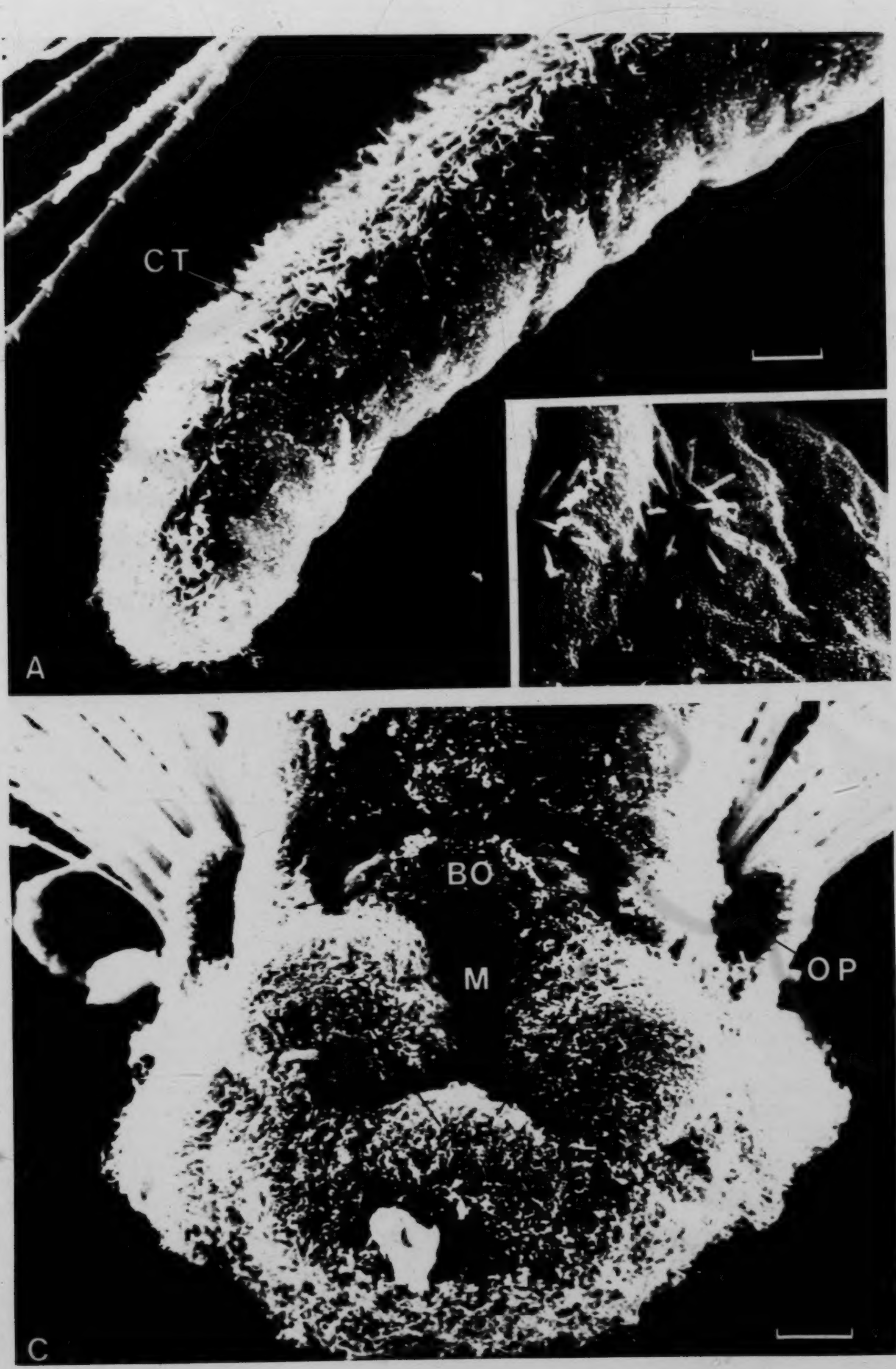

CT
A
BO
M
OP
C

Figure 12: SEM of a competent larva showing the uncini and setae.

A. Frontal view of the uncini of the abdominal uncinigerous lobes. Scale bar = 2 µm

B. View of the provisional setae showing the collar serrations which extend along the length of the setae. Scale bar = 4 µm

C. Dorsal view of the parathoracic segments showing the winged and capillary setae of the parapodial lobes. Note also the nototrochs on the posterior margins of the parathoracic segments. Scale bar = 10 µm

Legend:

CPS - Capillary setae
CS - Collar serrations
NT - Nototrochs
UL - Uncinigerous lobe
WS - Winged setae

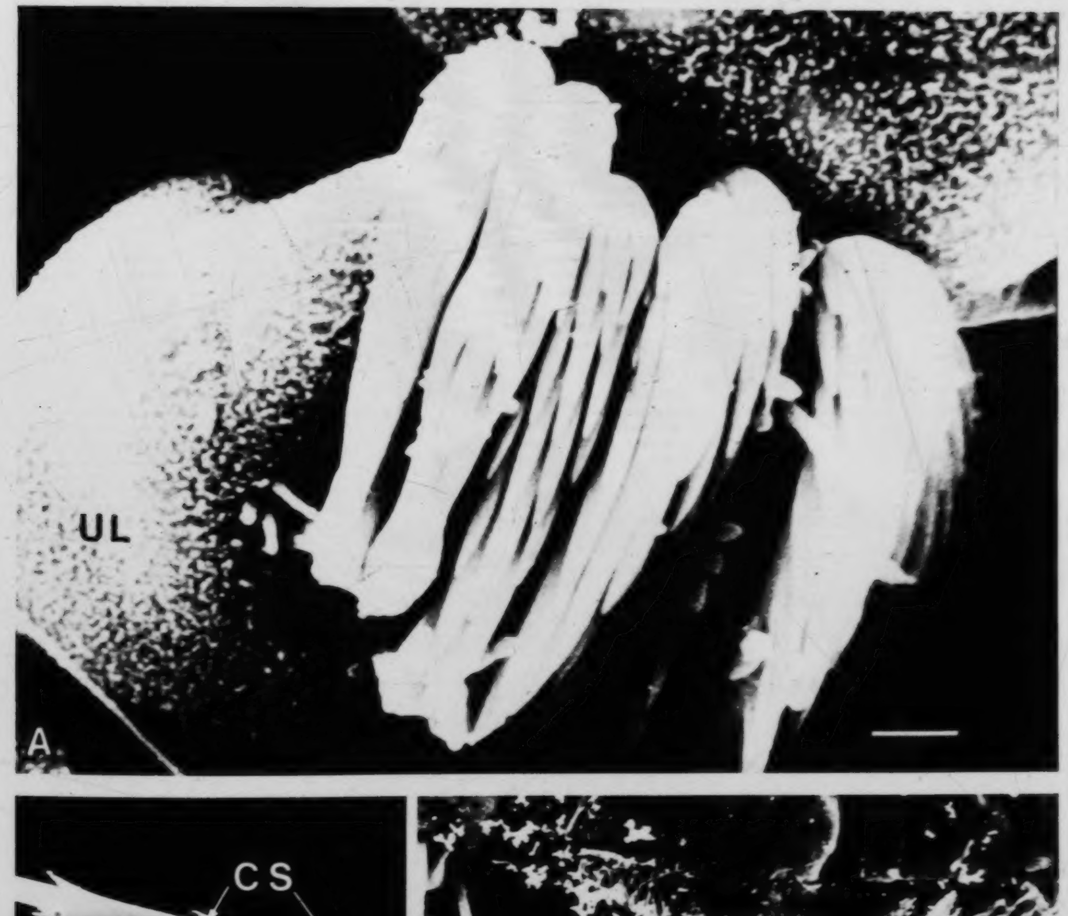

UL
A
CS
B
NT
WSL
CPS
C

Figure 13: SEM of a competent larva showing the epidermal gland pores.

A. Ventral surface of the parathoracic segments showing the numerous recessed gland pores. Ciliary sensory tufts can also be seen. Scale bar = 5 µm

B. Dorsal surface of a tentacle of the competent larva. The raised gland pore can be seen along with secretory material which was presumably discharged from the gland. Scale bar = 5 µm

Legend:

GP - Gland pore
NT - Neurotroch
S - Secretory material
ST - Sensory tuft

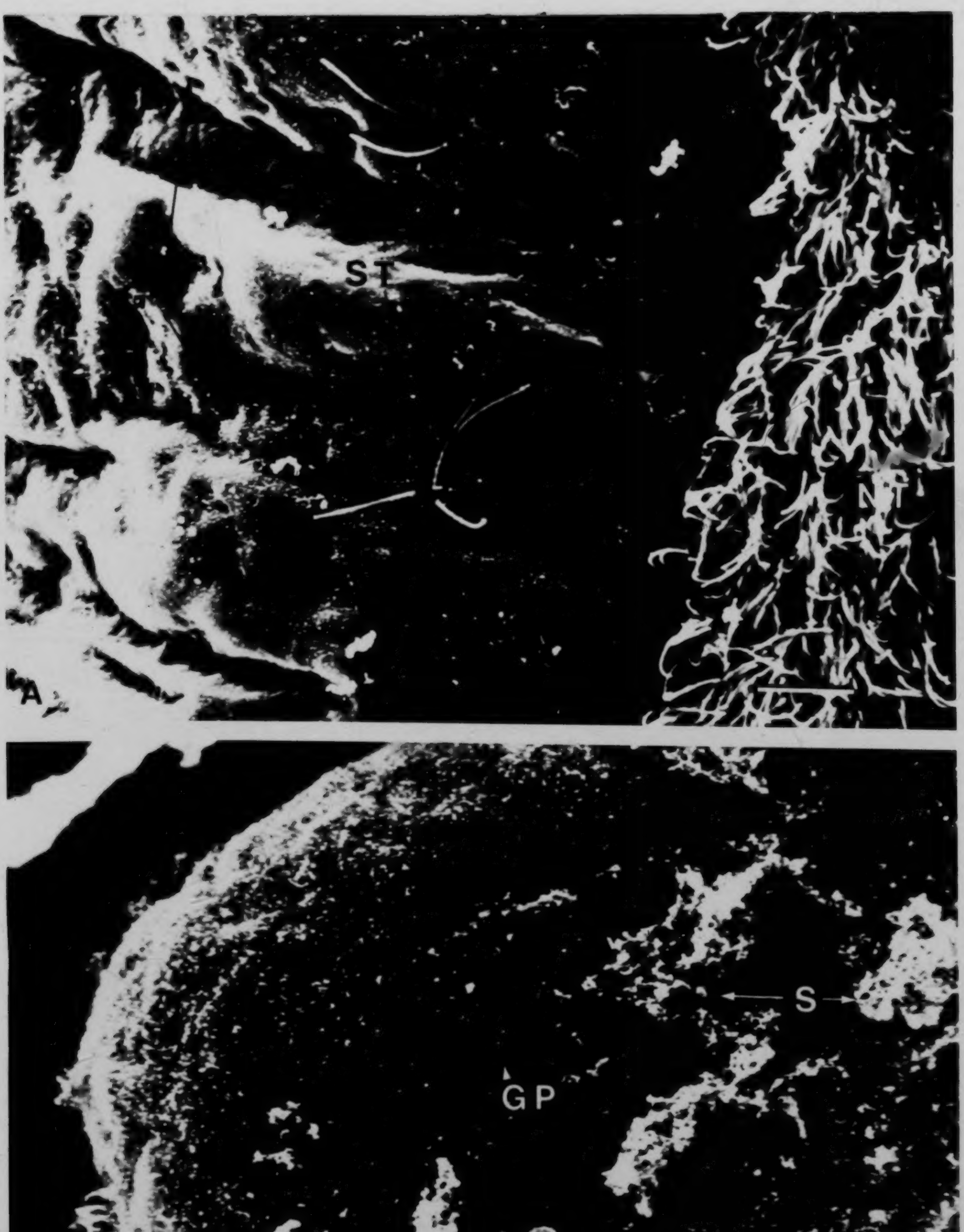

ST
NT
A
S
GP
B

Figure 14: SEM of episphere and pygidium of a competent larva.

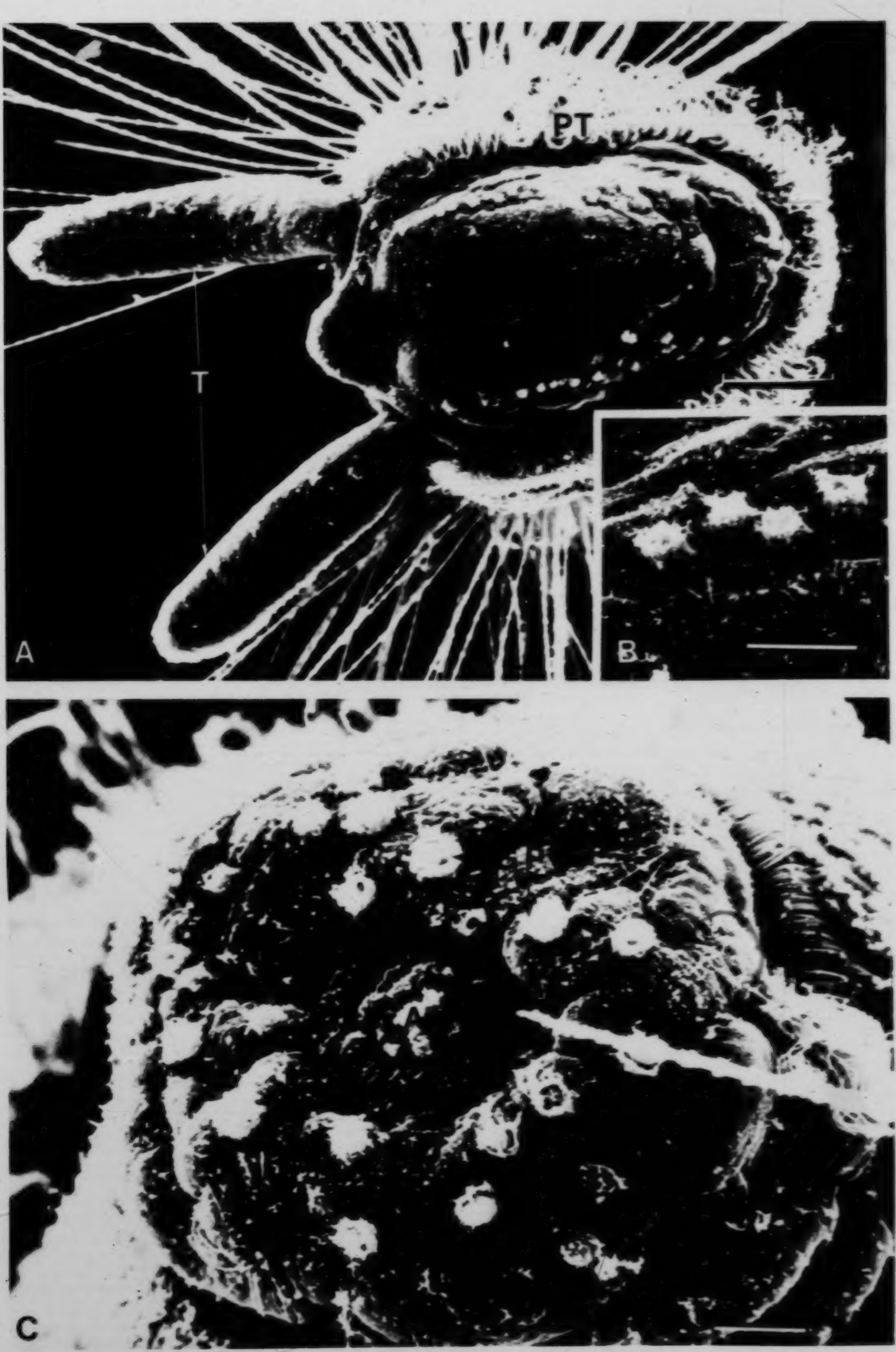
A. Apical view of the episphere showing the raised gland pores. Scale bar = 50 µm

B. Enlargement of epispheral gland pores. Scale bar = 10 µm

C. Posterior view of the pygidium showing the numerous raised gland pores surrounding the anal opening. Scale bar = 10 µm

Legend:

A - Anus
GP - Gland pores
PT - Prototroch
TT - Telotroch



7

Figure 15: Diagrammatical representation of the behavior of the competent and metamorphosing larvae of S. cementarium.

A. Competent larva exploring the sand grains with the top of its episphere. Arrows indicate the rotation of the larva.

B. Competent larva exploring the sand grains with its building organ.

C. Metamorphosing larva crawling over the substrate. The extended tentacle determines the direction of movement.

A

B

C

Figure 16: Defensive behavior of the planktonic larva.

A. Larva swimming with the provisional setae at the sides of the body prior to irritation. Scale bar = 40 µm

B. Larva which has been irritated. Note that the setae are erected to surround the body. Scale bar = 40 µm

Legend:

PS - Provisional setae

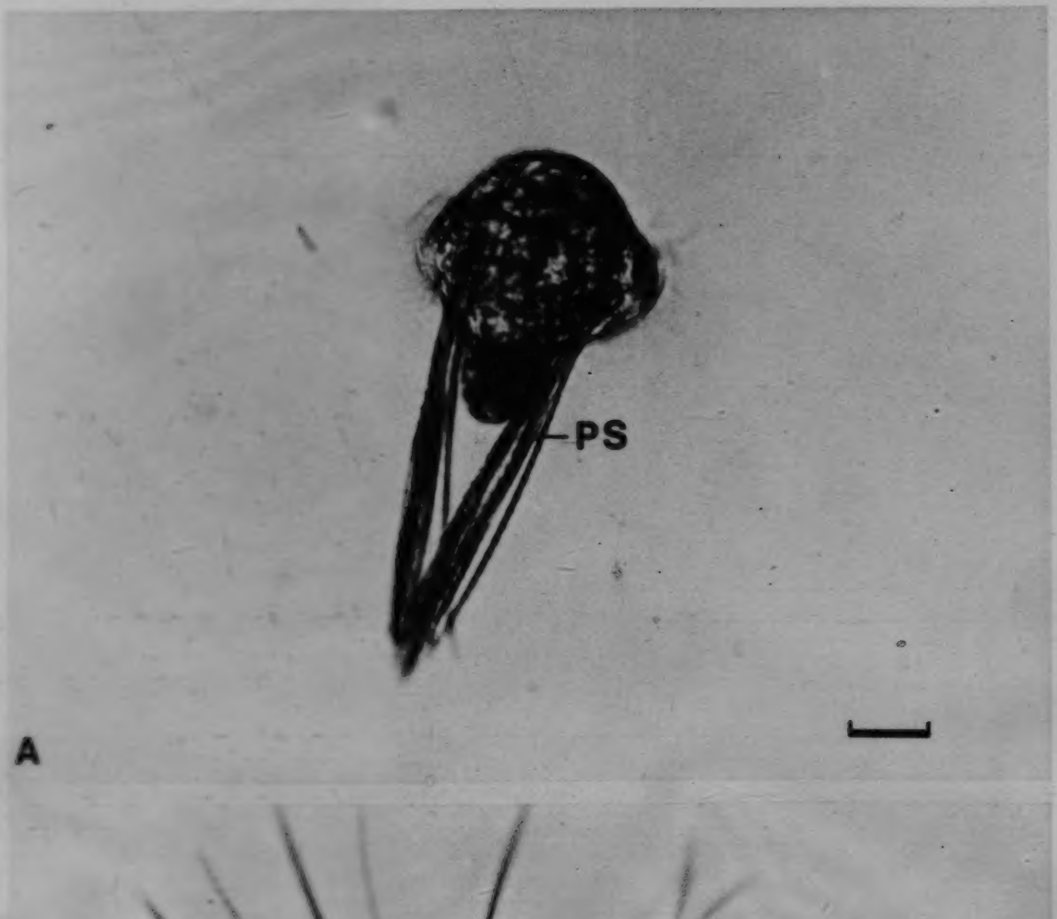

PS
A
PS
PS
B

Figure 17: Transmission electron micrographs (TEM) of the cuticle of a 5 day trochophore.

A. Section through the cuticle derived from the vitelline membrane showing Zones I and II. Note the branching microvilli extending from the epidermal cells. Scale bar = 0.05 µm

B. Section through the newly secreted larval cuticle. Note the absence of Zones I and II which were present in the cuticle derived from the vitelline membrane. The number of microvilli extending from the epidermal cells is reduced. Scale bar = 0.05 µm

Legend:

1 - Zone I
2 - Zone II
C - Cilium
L - Lipid droplet
MV - Microvilli

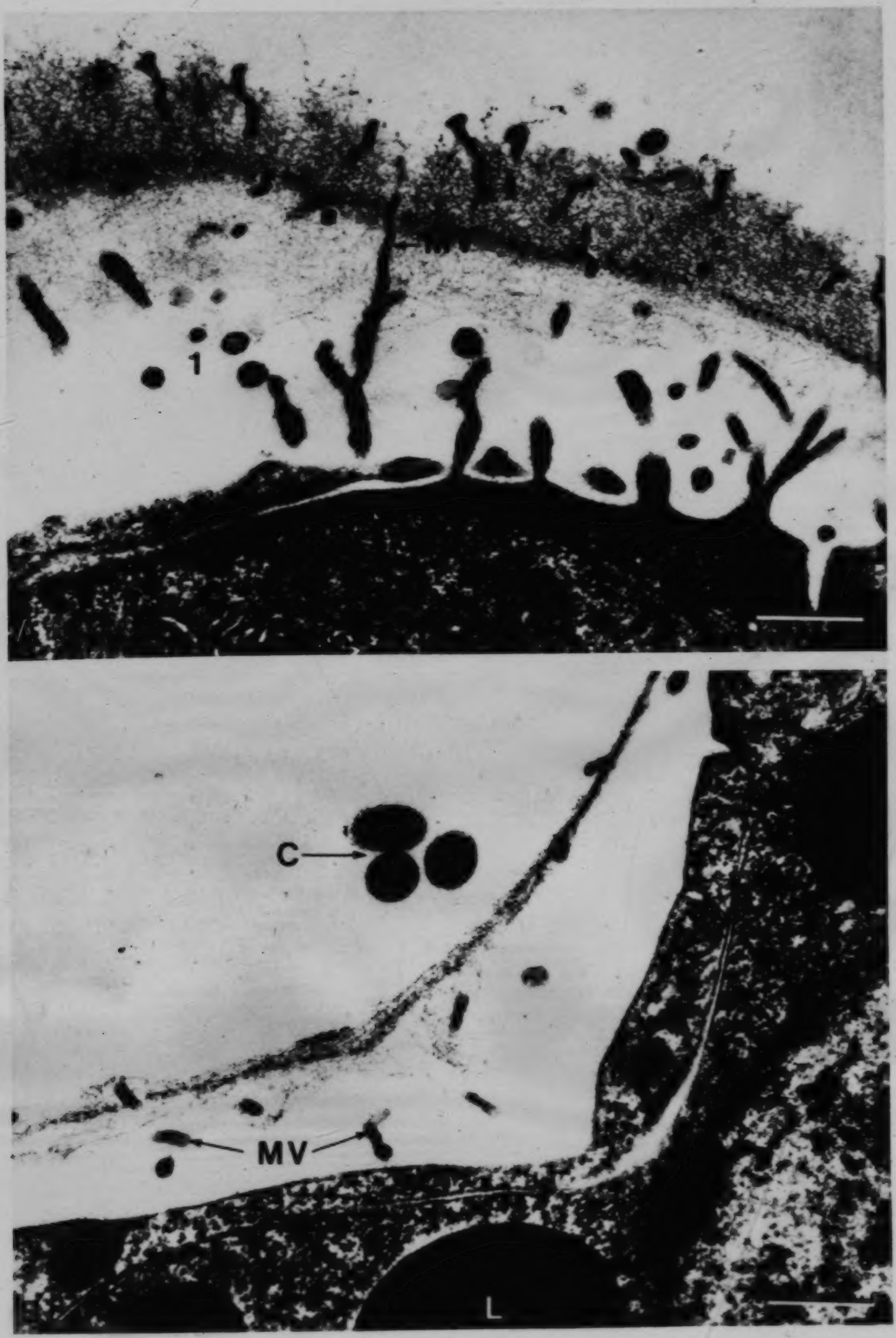

1
C
MV
L

Figure 18: TEM of prototrochal cells and esophagus of a 5 day trochophore.

A. Section through the prototroch showing the cuboidal shape of the prototrochal cell and the insertion of the prototrochal muscle below the prototrochal cell. Note the long, primary ciliary rootlets and the mitochondria within the prototrochal cell. Scale bar = 1 µm

B. Section through the esophagus showing the cuticular lining of the esophageal lumen. Note the numerous microvilli extending from the esophageal cells through the cuticle. Scale bar = 1 µm

Legend:

C - Cilia
CR - Ciliary rootlet
CU - Cuticle
L - Lipid droplet
MI - Mitochondria
M - Muscle

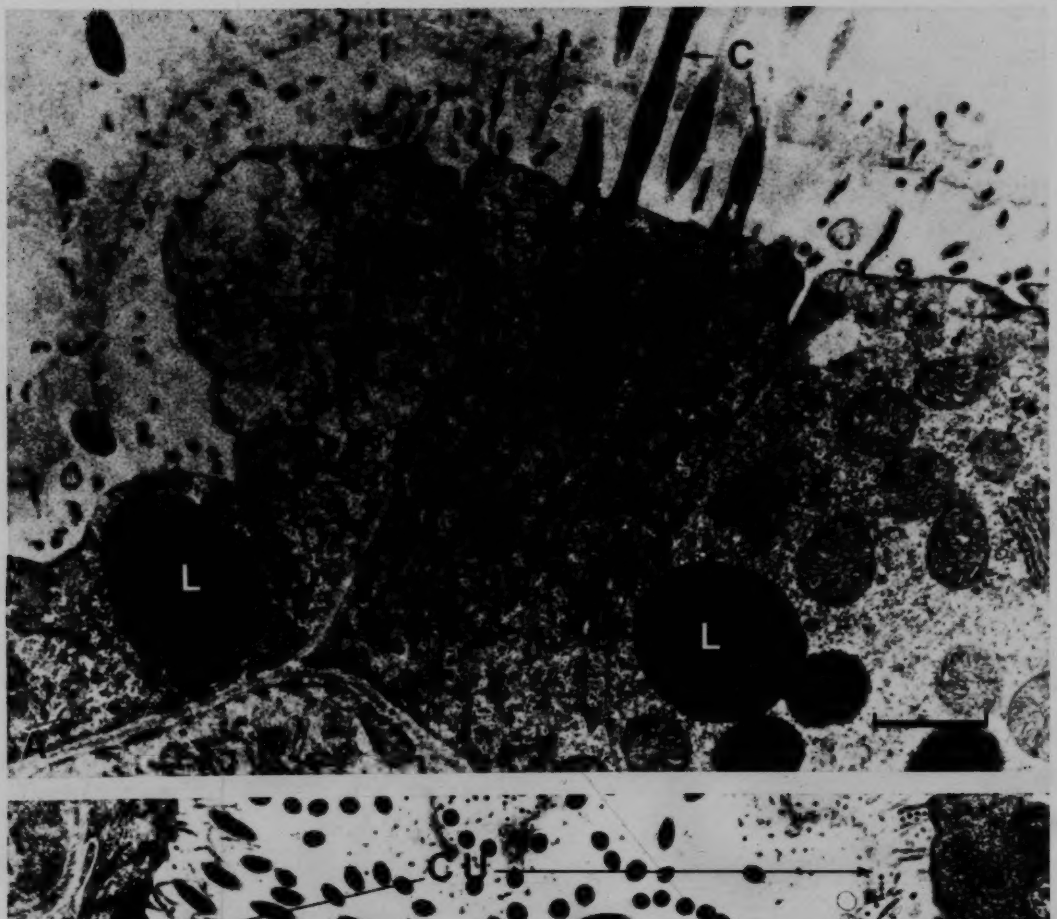

C
L
L
A
CU
C

Figure 19: Setal sacs.

A. Frontal section of a 46 hour trochophore showing a developing setal sac. At this stage the sac contains two chaetoblast cells. Note the developing provisional seta, indicated by the arrow. Scale bar = 5 µm

B. Sagittal section of a 65 hour trochophore. Note the increase in the number of cells comprising the setal sac. Scale bar = 5 µm

C. Sagittal section of a metatrochophore with tentacle buds showing the undifferentiated chaetoblast cells in the parapodia of the parathoracic segments.
Scale bar = 10 µm

D. Frontal section of a competent larva showing the parapodial setal sacs of the parathoracic segments. The chaetoblast cells are located basally in the sacs and they are indicated by the arrows. Scale bar = 10 µm

Legend:

CU - Cuticle
E - Esophagus
PS - Parathoracic setal sac
S - Seta
SS - Setal sac
ST - Stomach
TT - Telotroch
V - Vacuoles
VC - Vacuolated cells
VNC - Ventral nerve cord

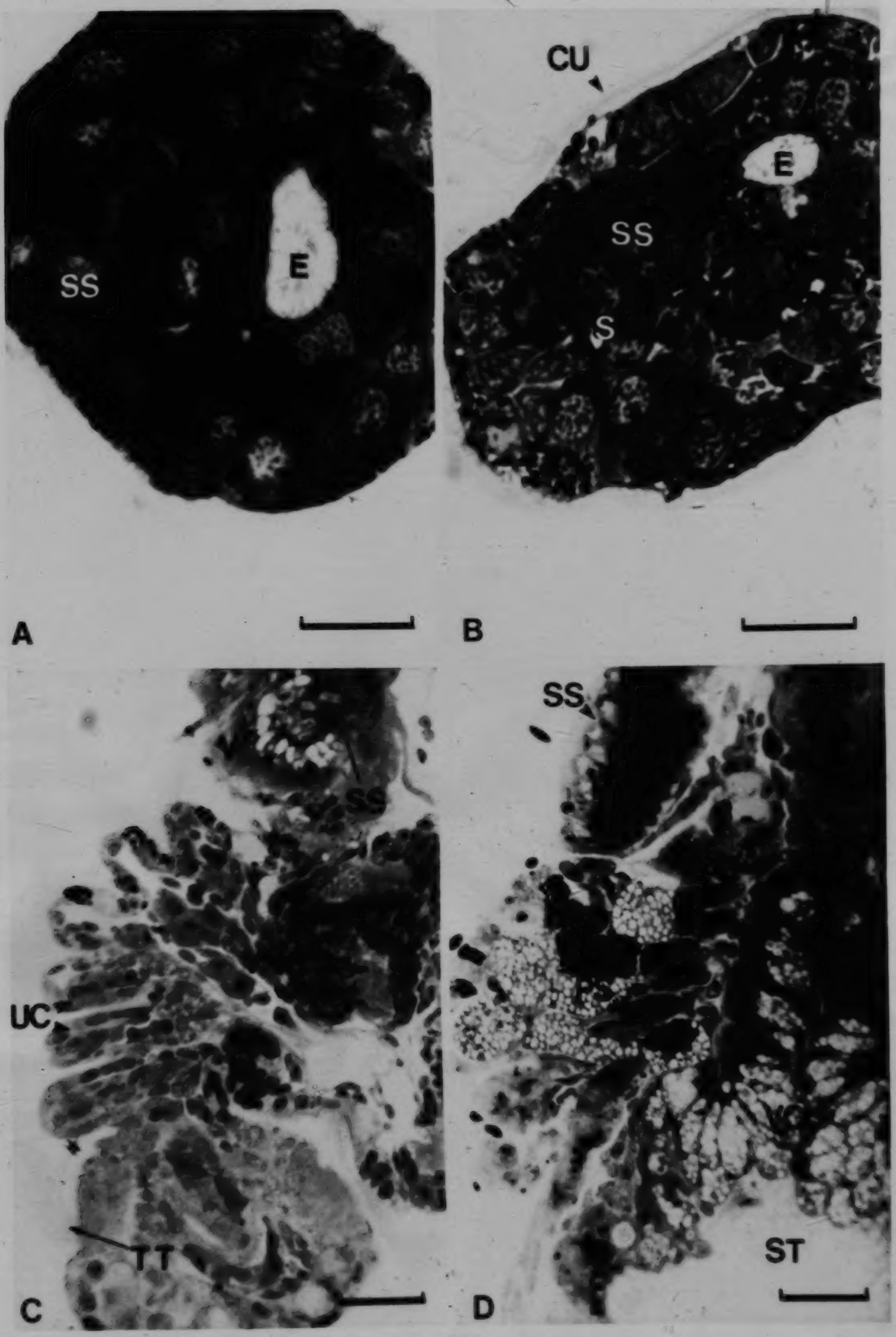

SS
E
CU
E
SS
S
A
B
SS
SS
UC
TT
C
D
ST

Figure 20: TEM of an oblique section through the setal sac of a 5 day trochophore.

Muscles invest the outer surface of the setal sac. Located within the setal sac are the lateral cells which secrete the cuticular lining of the follicle in which the provisional seta lies. Microvilli extend through the cuticle from the lateral cells. Note the microvilli at the base of the follicle on which the setae are formed. Scale bar = 0.25 µm

Legend:

CB - Chaetoblast cell
M - Muscle
MV - Microvilli
L - Lateral cell
S - Seta
V - Vacuole

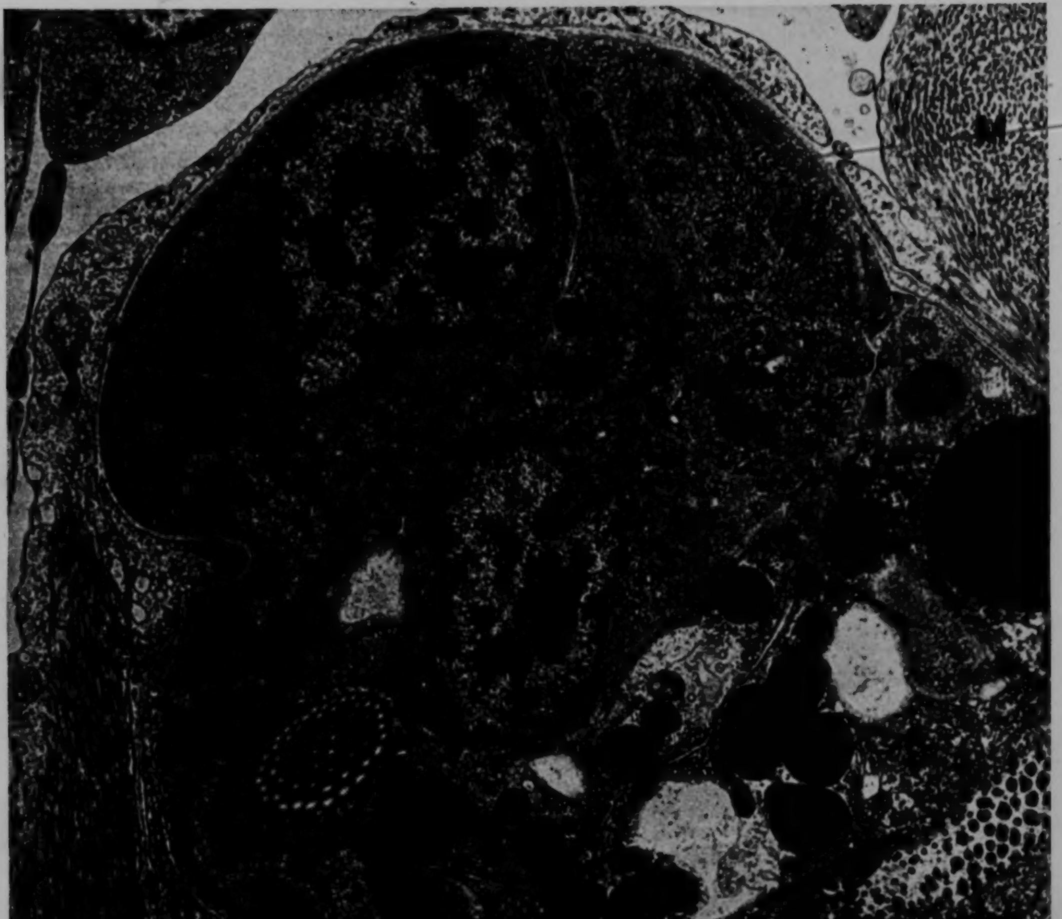

M
V
V

Figure 21: TEM of sections through the provisional setae of a 5 day trochophore.

A. Oblique section through a provisional seta showing the channels which extend the length of the seta. Scale bar = 0.1 µm

B. Section through the base of a follicle in which the provisional seta lies. Note the basal lamina lying between the muscle and the chaetoblast cell and the prominent microvilli of the chaetoblast cell on which the setae are formed. Scale bar = 0.1 µm

Legend:

BL - Basal lamina
C - Channel
M - Muscle
MV - Microvilli

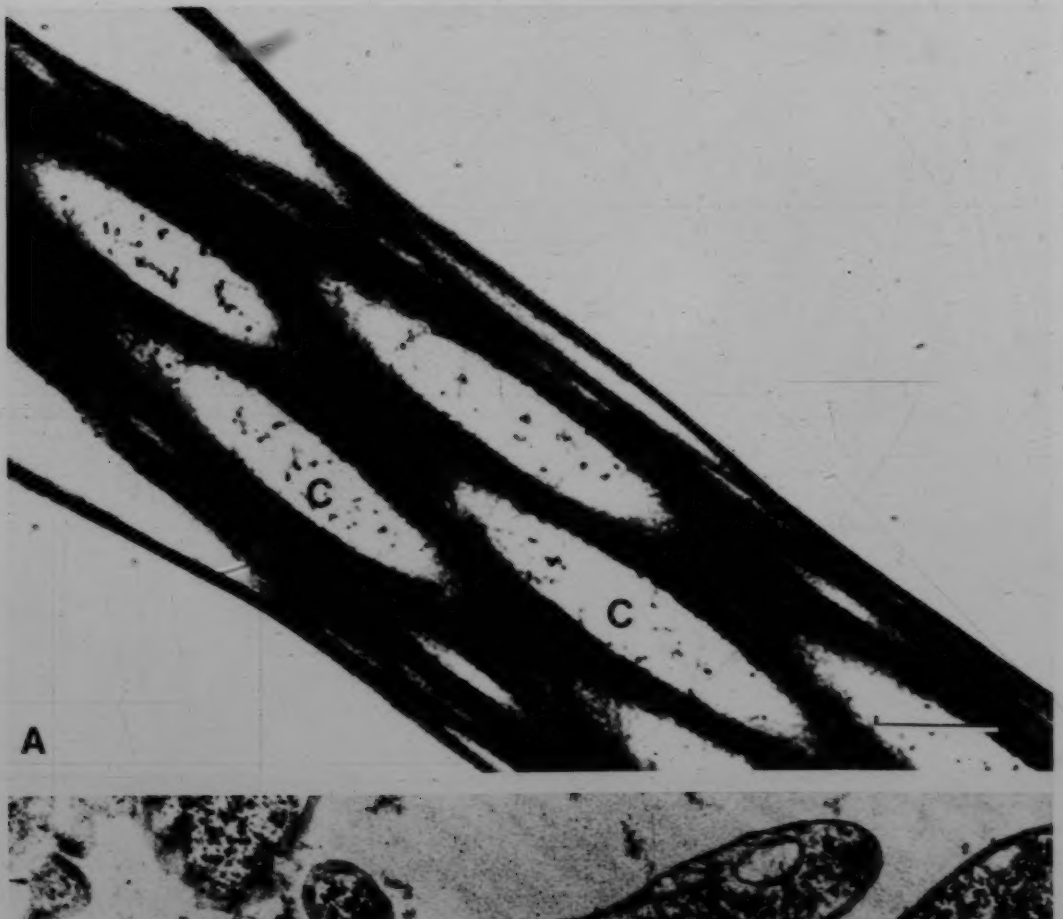

C
C
A
B

Figure 22: Development of muscle system, I.

A. Sagittal section of a 3.5 day trochophore showing the developing setal sac muscles which surround the setal sacs. Note the large cell containing numerous vacuoles in the posterior region of the esophagus. Scale bar = 5 µm

B. Frontal section of a 3.5 day trochophore showing the developing medial branch of the prototrochal muscle, which extends from the prototrochal cells to the setal sac muscles. Scale bar = 5 µm

C. Frontal section of a 5 day trochophore showing the prototrochal and esophageal muscles. Note the hypertrophy of the muscles relative to the 3.5 day trochophore. Scale bar = 10 µm

D. Frontal section of a metatrochophore with tentacle buds showing the supraesophageal and setal sac muscles. Note the circumesophageal blood vessel located within the peritoneum associated with the longitudinal muscles. Scale bar = 10 µm

Legend:

CEV - Circumesophageal blood vessel
E - Esophagus
EM - Esophageal muscle
PM - Prototrochal muscle
PMC - Presumptive mesodermal cells
PTC - Prototrochal cell
PTM - Prototrochal muscle
SEM - Supraesophageal muscle
SS - Setal sac
SSM - Setal sac muscle

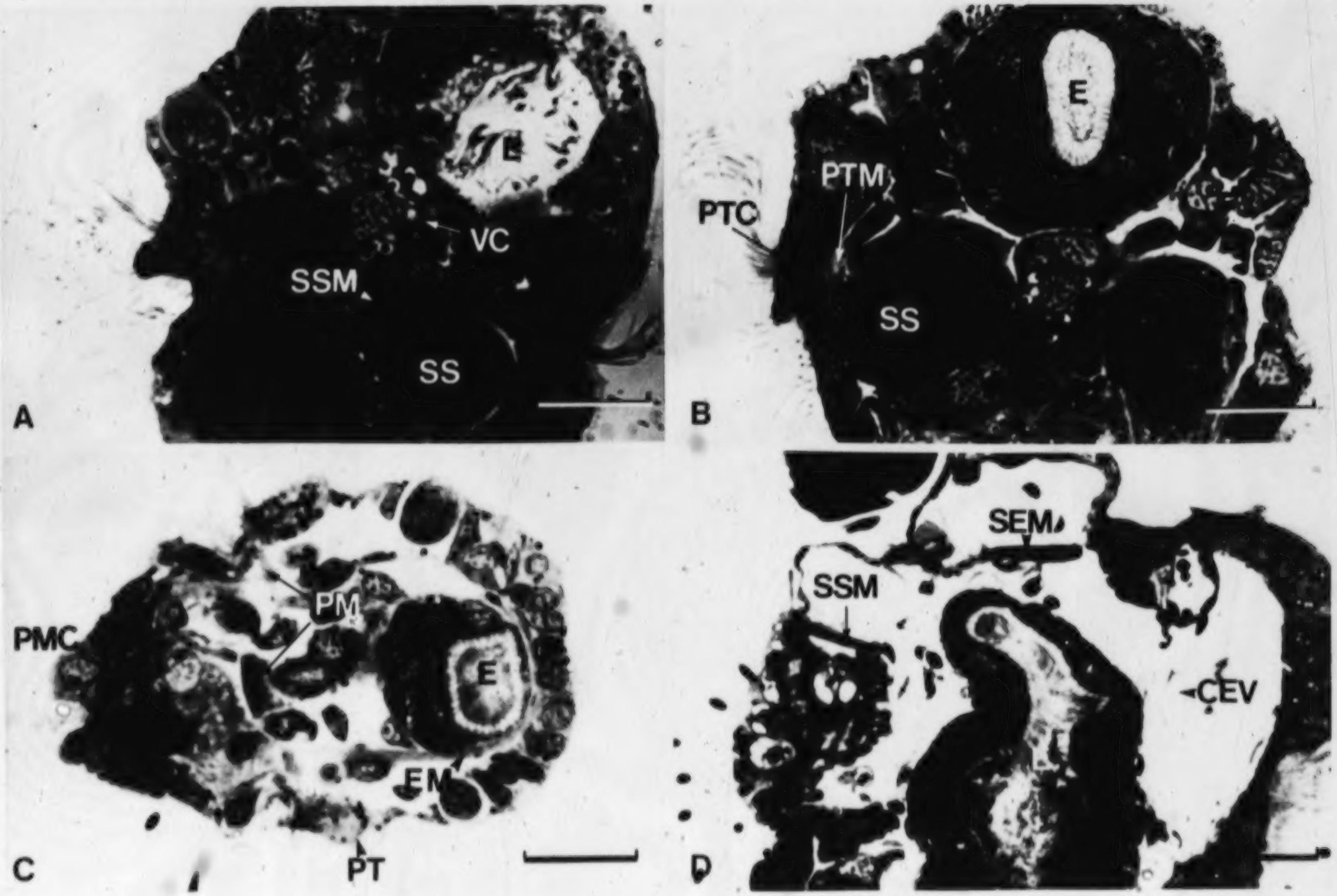

E
VC
SSM
SS
A
E
PTM
PTC
SS
B
PMC
PM
E
EM
PT
C
SEM
SSM
CEV
D

Figure 23: Development of muscle system, II.

A. Frontal section of a metatrochophore with tentacle buds showing the supraesophageal muscle which extends from the esophagus to the setal sac muscles. Note the posterior branch of the setal sac muscles which inserts onto the longitudinal muscles. Scale bar = 10 µm

B. Frontal section of a metatrochophore with tentacle buds showing the lateral muscle of the episphere which originates at the base of the tentacle and inserts onto the setal sac muscles. Scale bar = 10 µm

C. Frontal section of a metatrochophore with tentacle buds showing the medial branch of the prototrochal muscle. Scale bar = 10 µm

D. Frontal section of a competent larva showing the setal sac-esophageal muscle complex. Note the supraesophageal muscle and the posterior branch of the setal sac muscles which insert onto the setal sac and longitudinal muscles, respectively. Scale bar = 10 µm

Legend:

E - Esophagus
LM - Longitudinal muscles
MBPTM - Medial branch of the prototrochal muscle
SEM - Supraesophageal muscle
SS - Setal sac
SSM - Setal sac muscle
ST - Stomach
T - Tentacle

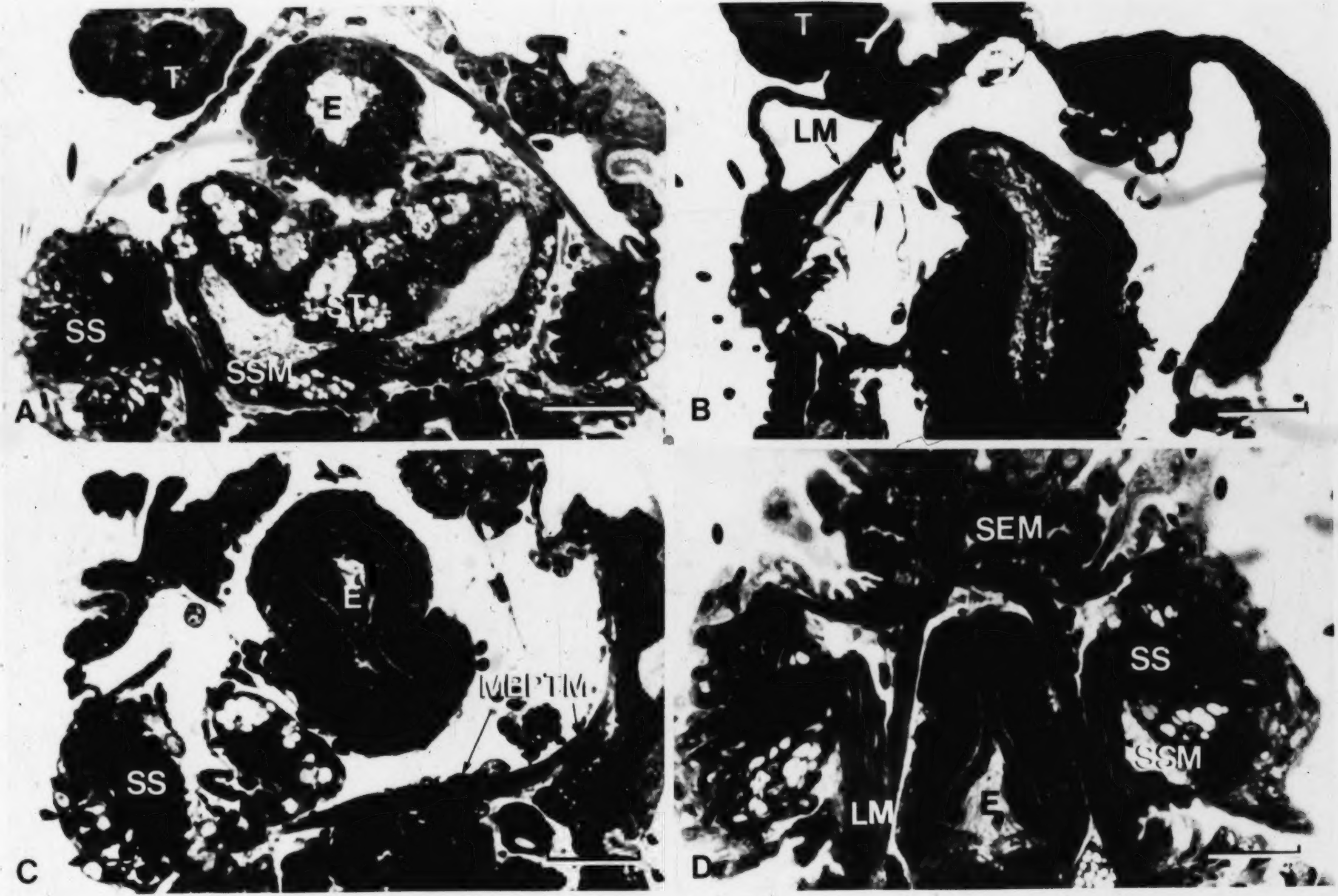

T
E
SS
ST
SSM
A
T
LM
E
B
E
SS
MBPTM
C
SEM
SS
SSM
LM
E
D

Figure 24: Development of muscle system, III.

A. Frontal section of a competent larva showing the esophageal connection of the setal sac muscles. Scale bar = 10 µm

B. Frontal section of a competent larva showing the medial branch of the prototrochal muscle. Note the ventral root of the circumesophageal commissure lying below the epidermis. Scale bar = 10 µm

C. Cross-section of a competent larva through the region of the intestine. Note that the dorsal longitudinal muscles appear larger than the ventral longitudinal muscles in cross-section. Scale bar = 10 µm

D. Cross-section of a competent larva showing the thin band of circular muscle lying below the epidermis. Scale bar = 5 µm

Legend:

CM - Circular muscle
DLM - Dorsal longitudinal muscle
E - Esophagus
ECSSM - Esophageal connection of the setal sac muscles
EG - Esophageal glands
MBPTM - Medial branch of the prototrochal muscle
NT - Neurotroch
SS - Setal sac
ST - Stomach
VLM - Ventral longitudinal muscle
VR - Ventral root of the circumesophageal commissure

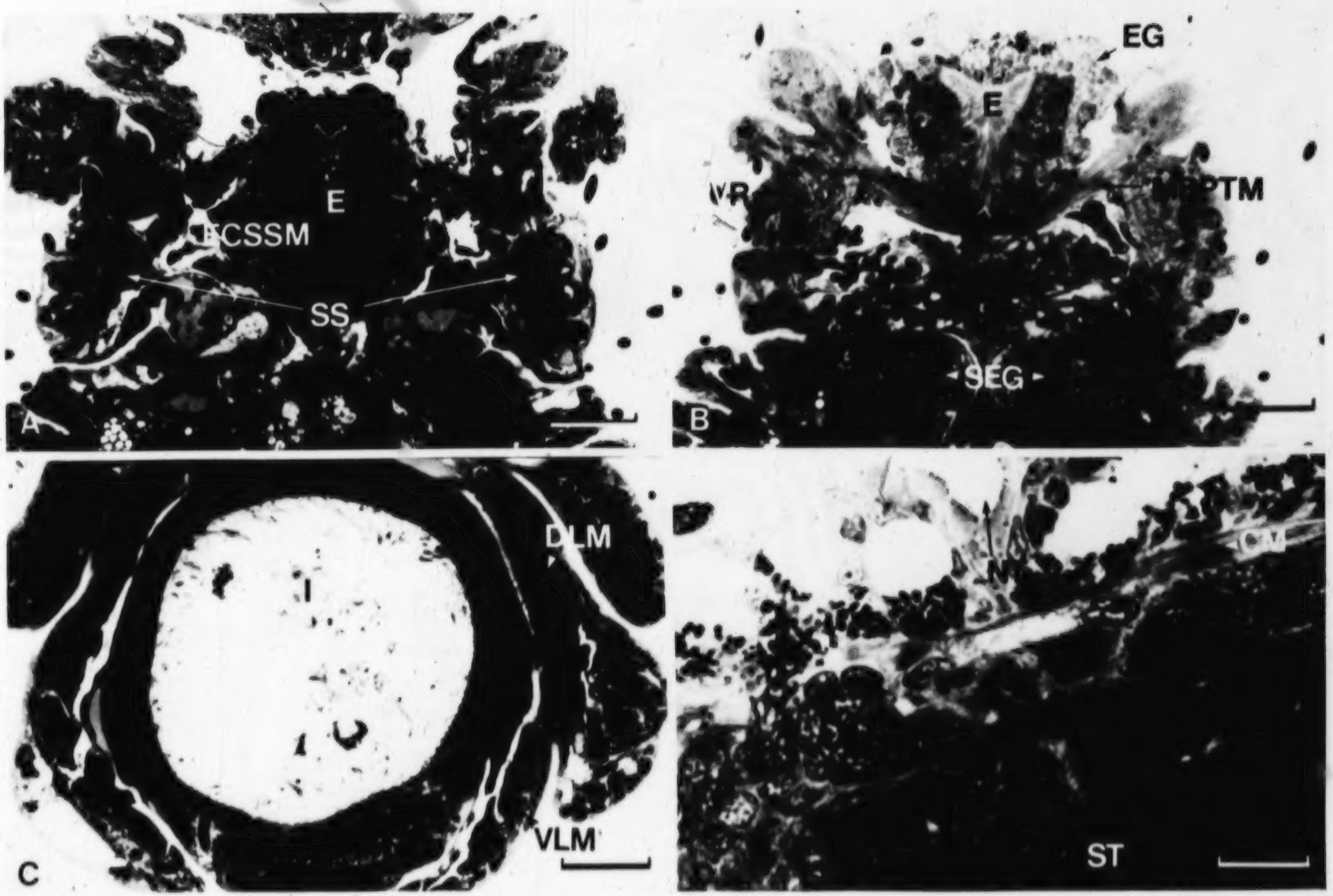

A
E
ECSSM
SS
B
EG
E
VR
PTM
SEG
C
DLM
I
VLM
N
CM
ST

Figure 25: Development of the coelomic cavities.

A. Frontal section of a 5 day trochophore showing the primary coelomic cavity. Note the presumptive mesodermal cells located in the hyposphere which will form the prepygidial growth zone in the later stage larvae. Scale bar = 10 µm

B. TEM section through the esophagus of a 5 day trochophore showing a portion of the peritoneum lying above the esophageal cells. Scale bar = 1 µm

C. Sagittal section of a metatrochophore with tentacle buds showing the primary coelomic cavity and the segmental coelomic cavities. Scale bar = 20 µm

D. Enlargement of the segmental coelomic cavities showing the thin septa which separate the coelomic cavities. Scale bar = 10 µm

Legend:

E - Esophagus
P - Peritoneum
PC - Primary coelomic cavity
PMC - Presumptive mesodermal cells
SC - Segmental coelomic cavity
SS - Setal sac
SSM - Setal sac muscles
ST - Stomach
VC - Vacuolated cells

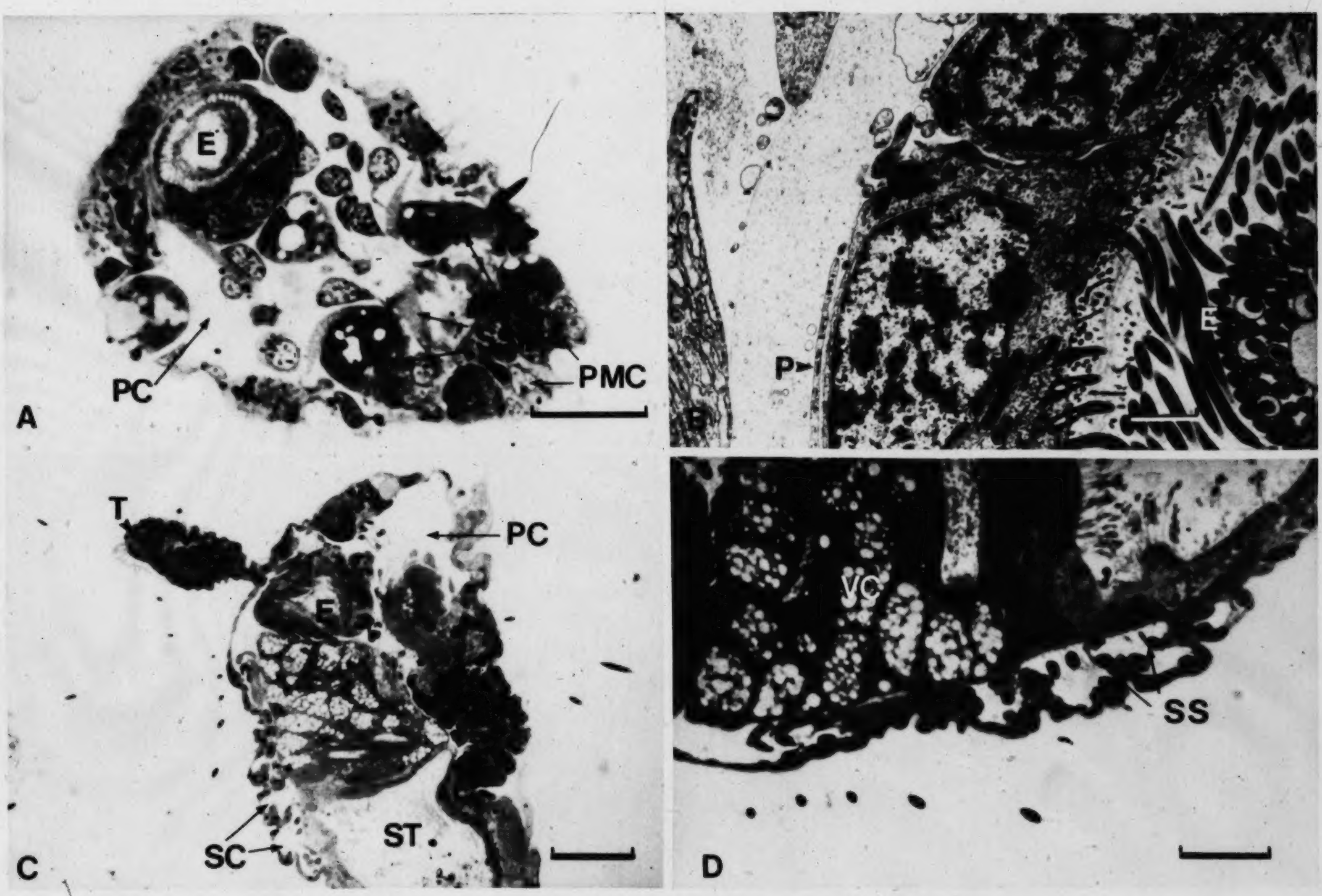

E
PC
PMC
A
P
E
B
T
PC
E
SC
ST
C
VC
SS
D

Figure 26: Development of alimentary tract, I.

A. Sagittal section of an 18 hour pretrochophore showing the numerous lipid droplets located within the cells and the rudiment of the lateral mucoid gland cell. Note the invagination which may represent the developing stomodeal invagination. Scale bar = 10 µm

B. Cross-section of a 23 hour trochophore showing the developing esophagus and stomach. Scale bar = 5 µm

C. Sagittal section of a 44 hour trochophore showing the stomodeal invagination and stomach. Scale bar = 5 µm

D. Frontal section of a 44 hour trochophore showing the esophagus and stomach. Note the concentration of lipid droplets within the esophageal cells and the sparse ciliation of the stomach cells. Scale bar = 5 µm

Legend:

E - Esophagus
I - Stomodeal invagination
L - Lipid droplets
MC - Mucoid gland cell
PT - Prototrochal cell
ST - Stomach

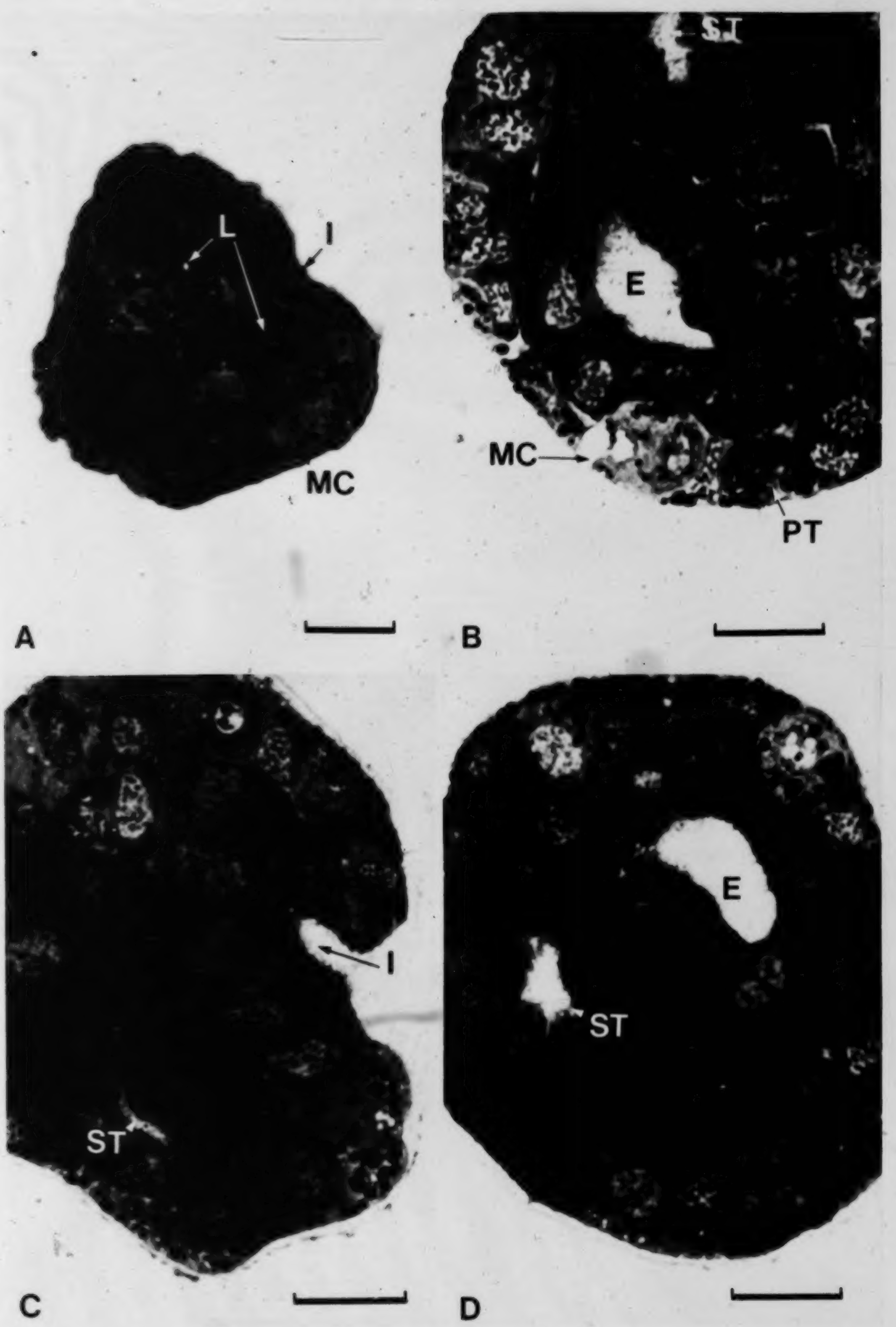

L
I
MC
A
ST
E
MC
PT
B
I
ST
C
E
ST
D

Figure 27: Development of alimentary tract, II.

A. Sagittal section of 65 hour trochophore showing the columnar esophageal cells. Note the setal sacs and paired, lateral mucoid gland cells. Scale bar = 5 µm

B. Sagittal section of a 65 hour trochophore showing the large vacuolated cell of the postero-lateral portion of the esophagus. Scale bar = 5 µm

C. Cross-section of a 65 hour trochophore showing the esophagus and stomach. Note the reduction in number of the lipid droplets within the esophageal and stomach cells. Scale bar = 5 µm

D. Frontal section of a 3.5 day trochophore showing the elongation of the esophagus. Arrows indicate the cuticular lining of the esophageal lumen. Scale bar = 5 µm

Legend:

E - Esophagus
MG - Mucoid gland cells
PT - Prototrochal cell
S - Seta
SS - Setal sac
ST - Stomach

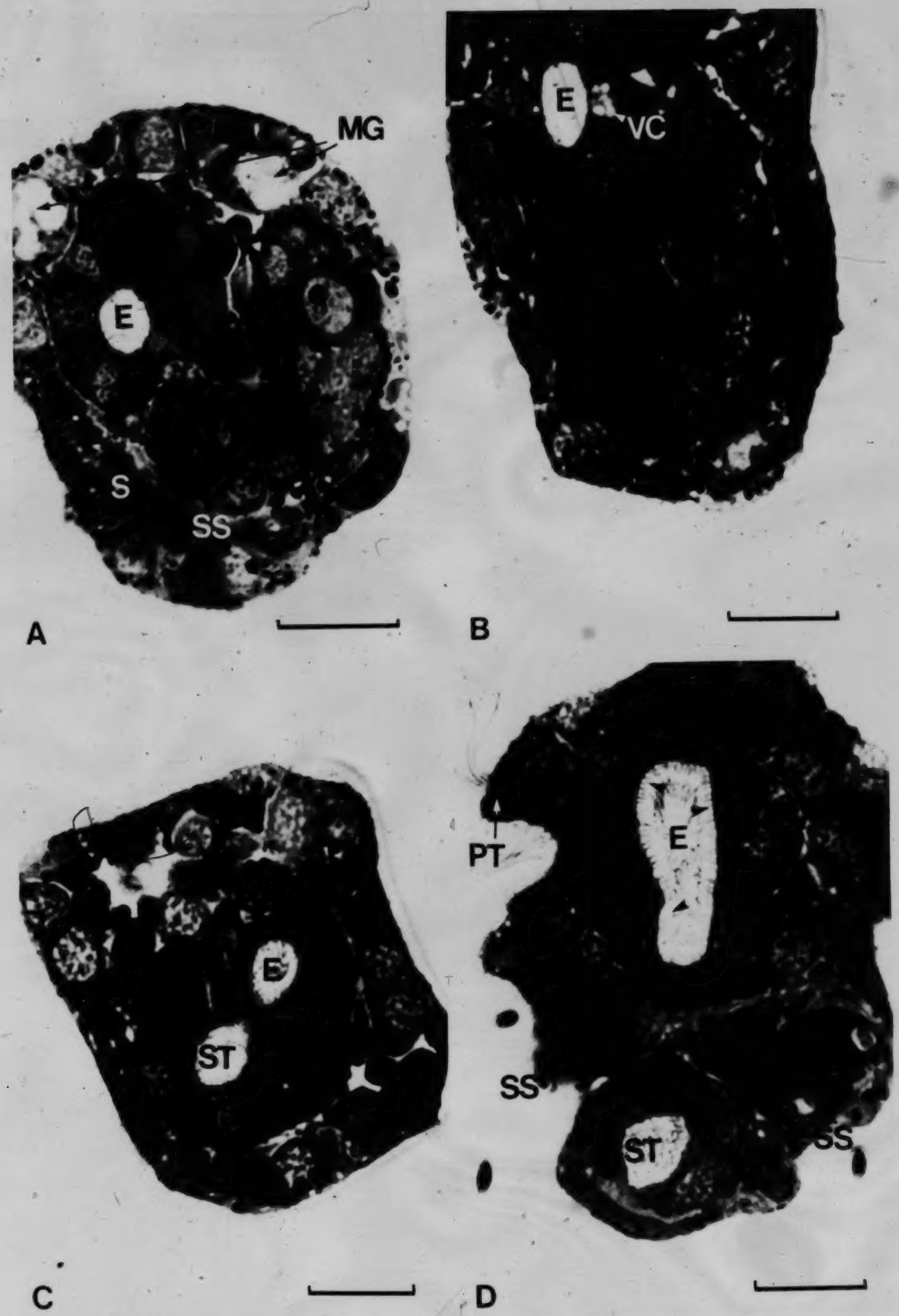

MG
E
S
SS
A
E
VC
B
E
ST
C
PT
E
SS
ST
SS
D

Figure 28: Development of alimentary tract, III.

A. Sagittal section of a 5 day trochophore showing the septum which demarcates the stomach and intestine. Scale bar = 20 µm

B. Frontal section of a metatrochophore. The arrows indicate the dense border of cilia within the lumen of the esophageal-stomach junction. Scale bar = 5 µm

C. Sagittal section of a metatrochophore showing the vacuolated cells of the upper stomach. Note the elongation of the stomach and the presence of numerous lipid droplets within the stomach cells. Scale bar = 10 µm

D. Cross-section of a metatrochophore through the region of the stomach. Note the stomach cells possessing presumptive zymogen granules. Scale bar = 5 µm

Legend:

E - Esophagus
I - Intestine
L - Lipid droplets
PZG - Presumptive zymogen granules
S - Septum
SS - Setal sac
ST - Stomach
VC - Vacuolated cells

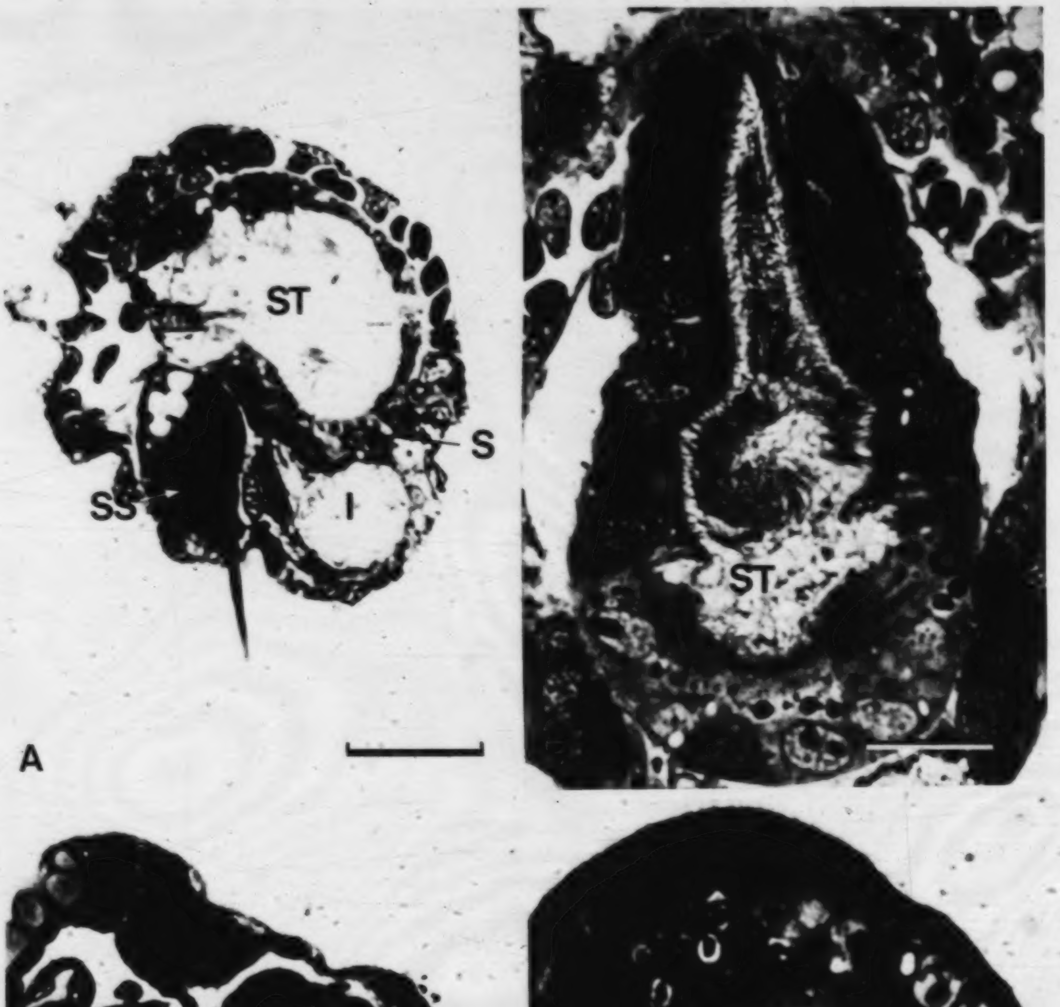

ST
S
SS
I
A
ST
SS
VC
ST
L
C
ST
PZG
D

Figure 29: Development of alimentary tract, IV.

A. Frontal section of a metatrochophore with tentacle buds showing the light and dark-staining cells of the esophagus. Scale bar = 10 µm

B. Frontal section of a metatrochophore with tentacle buds showing the rudiments of the esophageal glands. Scale bar = 5 µm

C. Frontal section of a competent larva showing the esophageal gland cells which contain the pink-staining flocculent material. Note the medial branch of the prototrochal muscle and the ventral ganglia. Scale bar = 10 µm

D. Frontal section of a competent larva showing the vacuolated cells of the stomach. Note the thin septum separating the stomach and intestine and the weakly ciliated cells of the intestine. Scale bar = 10 µm

Legend:

DC - Dark-staining cells of the esophagus
E - Esophagus
EG - Esophageal glands
LC - Light-staining cells of the esophagus
M - Medial branch of the prototrochal muscle
S - Septum
ST - Stomach
VC - Vacuolated cells
VG - Ventral ganglia

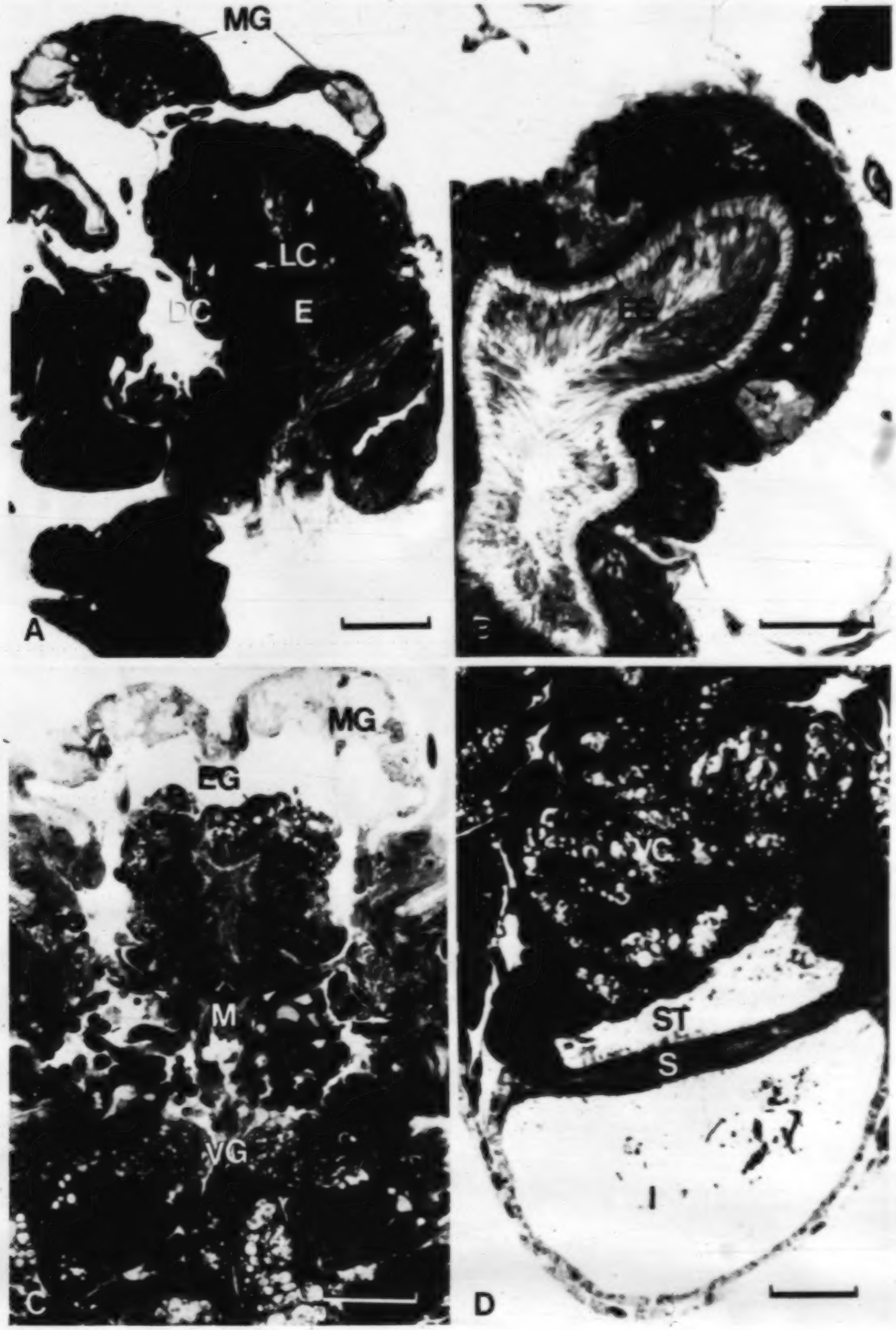

MG
LC
DC
E
A
ES
B
MG
EG
M
VG
C
VC
ST
S
I
D

Figure 30: TEM of the stomach of a 5 day trochophore.

A. Section through the stomach showing the vacuolated cells of the upper stomach. Note the presence of microvilli on the vacuolated cells. The lower esophagus can be seen in the upper right. Scale bar = 5 µm

B. Enlargement of the vacuolated cells of the upper stomach. Arrows indicate the vacuoles which enclose a flocculent material. Scale bar = 0.25 µm

Legend:

E - Esophagus
MV - Microvilli
ST - Stomach
VC - Vacuolated cell

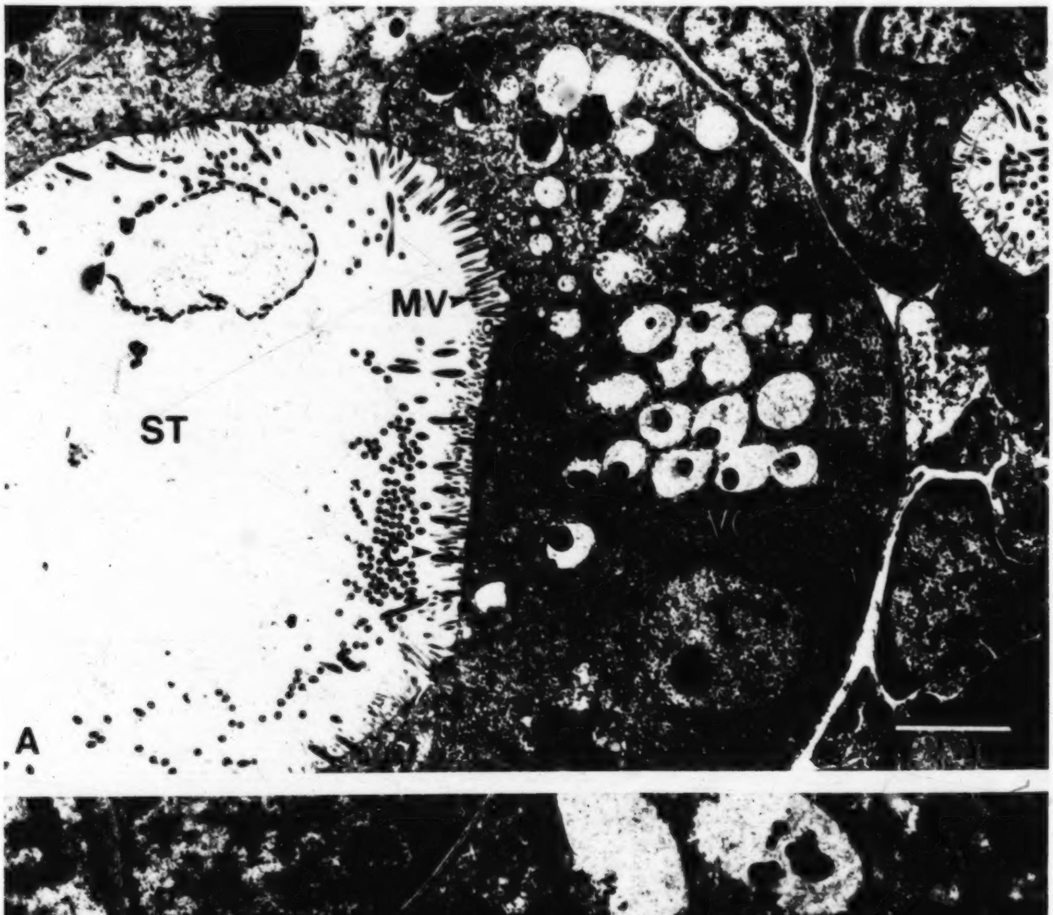

MV
ST
VC
A
B

Figure 31: Development of nervous system, I.

A. Frontal section of a 12 day trochophore showing the cerebral ganglion. Scale bar = 10 µm

B. Frontal section of a metatrochophore showing the dorsal root of the circumesophageal commissure and the subesophageal ganglion. Scale bar = 10 µm

C. Frontal section of a metatrochophore showing the cerebral ganglion which is now bilobed. Note the marked enlargement of this ganglion. Scale bar = 5 µm

D. Frontal section of a metatrochophore showing the developing pigment-cup eyespot and the ventral root of the circumesophageal commissure. Scale bar = 5 µm

Legend:

CG - Cerebral ganglion
DCG - Dorsal root of the circumesophageal commissure
E - Esophagus
EY - Eyespot
MG - Mucoid gland cell
PT - Prototrochal cell
SEG - Subesophageal ganglion
ST - Stomach
VC - Vacuolated cells
VCG - Ventral root of the circumesophageal commissure

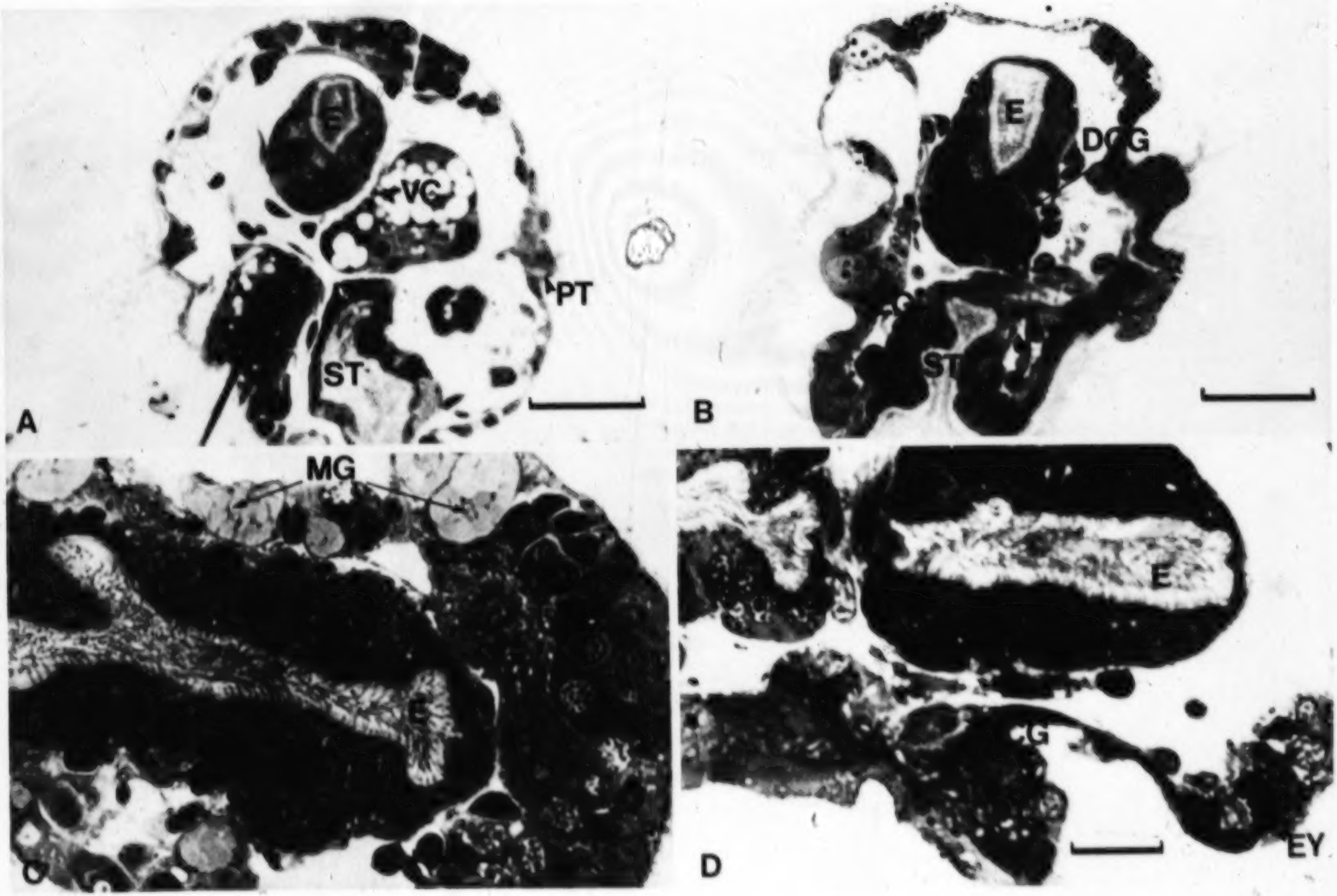

E
VG
PT
ST
A
E
DCG
ST
B
MG
E
C
E
G
EY
D

Figure 32: Development of nervous system, II.

A. Frontal section of a metatrochophore with tentacle buds showing the dorsal and ventral roots of the circumesophageal commissure. Note the ventral nerve cord which first becomes apparent in this stage. Scale bar = 10 μm

B. Frontal section of a metatrochophore with tentacle buds showing the subesophageal ganglion. Note the enlargement of this ganglion. Scale bar = 10 μm

C. Sagittal section of a metatrochophore with tentacle buds showing the subesophageal ganglion. Note the rudiments of gland cells A, B, and C. Scale bar = 10 μm

D. Frontal section of a competent larva showing the bilobed cerebral ganglion. Scale bar = 10 μm

Legend:

CG - Cerebral ganglion
DR - Dorsal root of the circumesophageal commissure
E - Esophagus
gAB - Rudiment of gland cells A and B
gC - Rudiment of gland cell C
SEG - Subesophageal ganglion
ST - Stomach
VNC - Ventral nerve cord
VR - Ventral root of the circumesophageal commissure

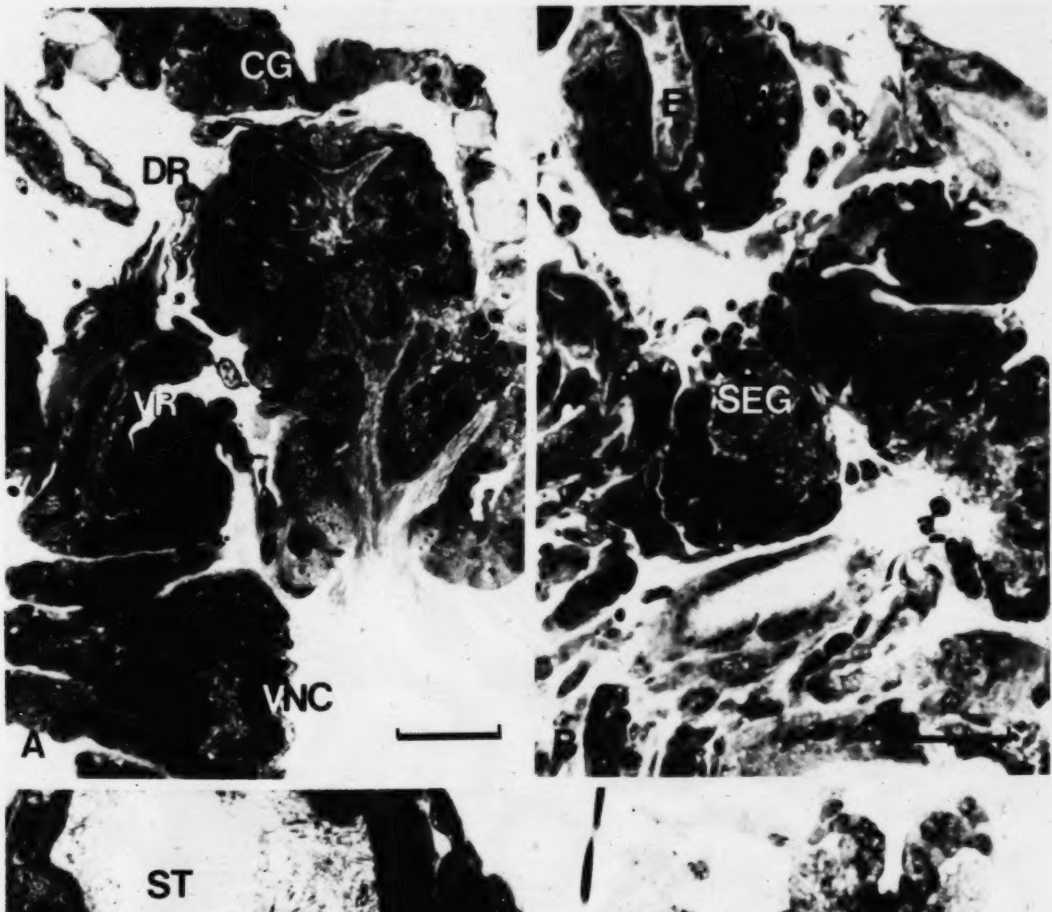

CG
DR
VR
VNC
A
E
SEG
B
ST
gAB
gC
C
SEG
CG
E
D

Figure 33: Development of nervous system, III.

A. Sagittal section of a competent larva showing the cerebral ganglion, eyespot, and apical sensory cell. Arrows indicate the connectives from the cerebral ganglion to the eyespot and sensory cell. Scale bar = 5 µm

B. Sagittal section of a competent larva showing the positions of the dorsal and ventral roots of the circumesophageal commissures in relation to the cerebral ganglion. Scale bar = 5 µm

C. Frontal section of a competent larva showing the dorsal and ventral roots of the circumesophageal commissure, subesophageal ganglion, and ventral ganglia. Note the glandularity of the building organ. Scale bar = 10 µm

D. Cross-section of a competent larva showing the paired ventral nerve cords. Note the numerous lipid droplets within the stomach cells. Scale bar = 10 µm

Legend:

AT - Apical sensory cell
BO - Building organ
CG - Cerebral ganglion
DR - Dorsal root of the circumesophageal commissure
E - Esophagus
EY - Eyespot
gB - Gland cell type B
L - Lipid droplets
LM - Longitudinal muscles
MG - Mucoid gland cells
SEG - Subesophageal ganglion
VG - Ventral ganglia
VNC - Ventral nerve cord
VR - Ventral root of the circumesophageal commissure

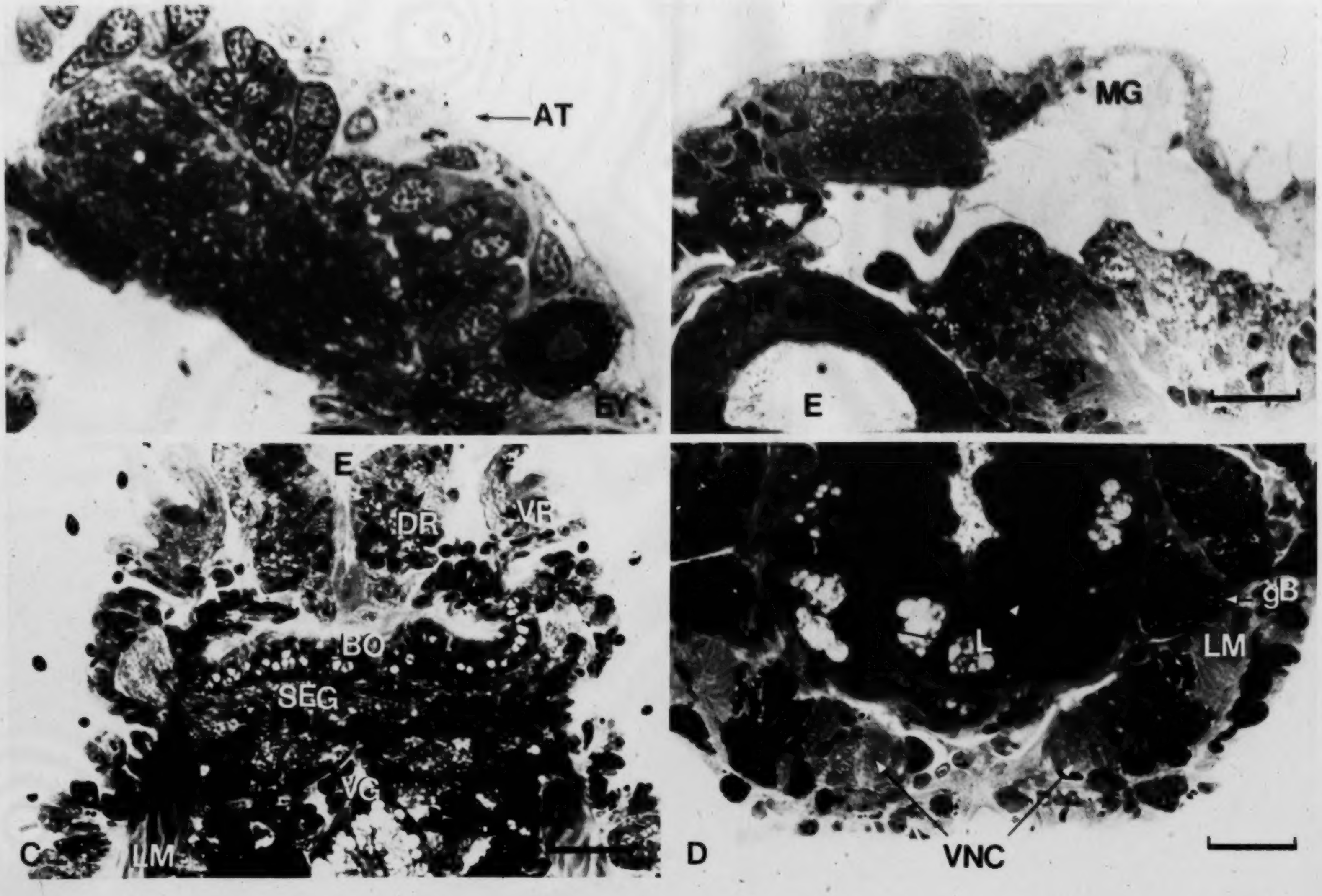

AT
MG
EY
E
E
DR
VR
BO
SEG
VG
C
LM
D
gB
LM
L
VNC

Figure 34: Circulatory system.

A. Sagittal section of a metatrochophore showing the developing supraesophageal and circumesophageal blood vessels. Note the mucoid gland cells which extend from the episphere to the pygidium. Scale bar = 20 µm

B. Frontal section of a competent larva showing the supraesophageal blood vessel. Scale bar = 10 µm

C. Cross-section of a competent larva showing the dorsal blood vessel lying above the supraesophageal muscle. Note the thin membrane surrounding the lumen of the blood vessel and the prominent nucleus of the membrane. Scale bar = 10 µm

D. Cross-section of a competent larva showing the dorsal and ventral blood vessels. Scale bar = 10 µm

Legend:

CEV - Circumesophageal blood vessel
DS - Dorsal blood sinus
DV - Dorsal blood vessel
E - Esophagus
EY - Eye
LM - Longitudinal muscle
MG - Mucoid gland cell
SEM - Supraesophageal muscle
SEV - Supraesophageal blood vessel
SS - Setal sac
ST - Stomach
VS - Ventral blood sinus

1

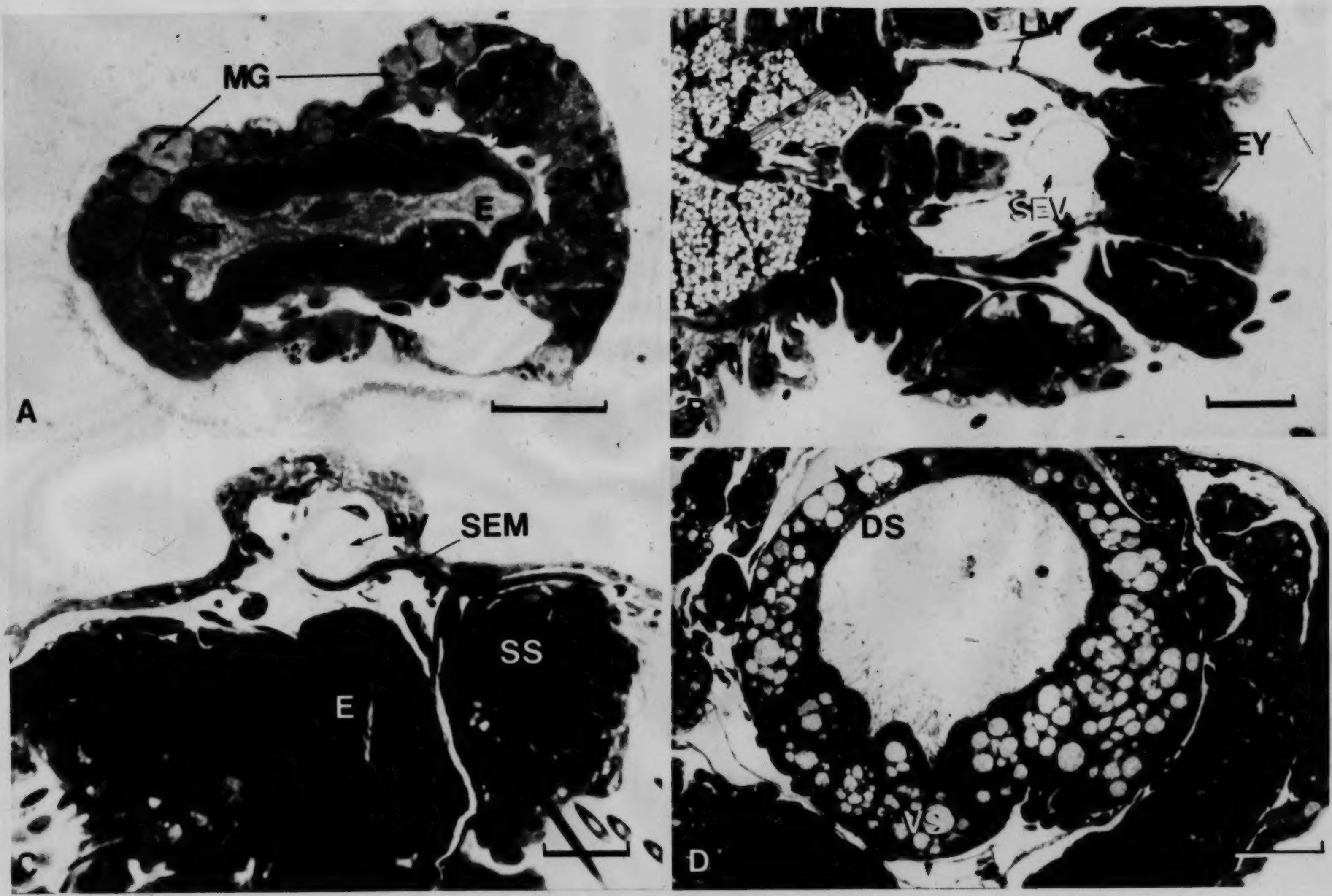


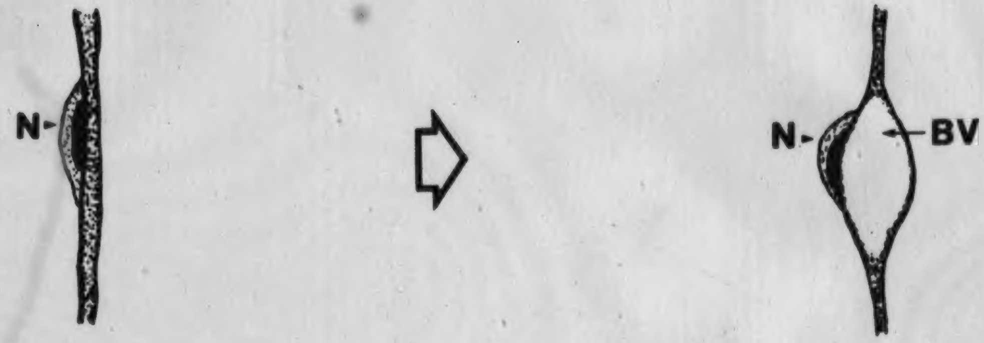
Figure 35: Diagrammatical representation of blood vessel formation.

A. Formation of a blood vessel by the separation of the apposed surfaces of a mesentery.

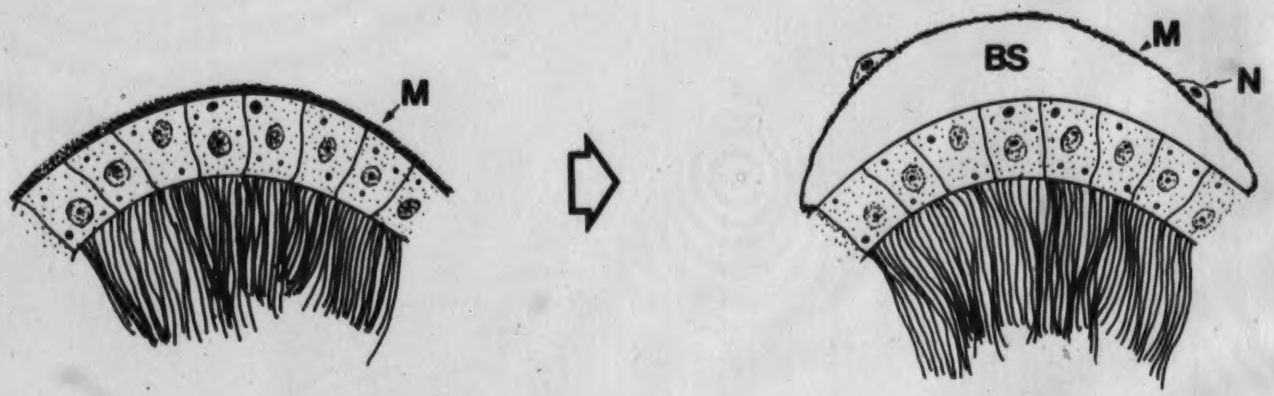
B. Formation of a blood sinus by the separation of the mesoderm from the wall of the gut.

Legend:

BS - Blood sinus
BV - Blood vessel
M - Mesoderm
N - Nucleus



A



B

Figure 36: Development of gland cells, I.

A. Cross-section of a 23 hour trochophore showing the developing lateral, unicellular mucoid gland located above the prototroch. Scale bar = 5 µm

B. Cross-section of a 65 hour trochophore showing the further development of the lateral mucoid gland. Note the increase in the amount of secretory material within the gland. Scale bar = 5 µm

C. Frontal section of a 3.5 day trochophore. An apical mucoid gland, situated above the esophagus, first appears in this stage. Note the well developed setal sacs. Scale bar = 5 µm

D. Frontal section of a 3.5 day trochophore showing the enlargement of the lateral mucoid gland. Note the prominent vacuolated cell located in the upper stomach. Scale bar = 5 µm

Legend:

E - Esophagus
MG - Mucoid gland
PTC - Prototrochal cell
SS - Setal sac
ST - Stomach
VC - Vacuolated cell

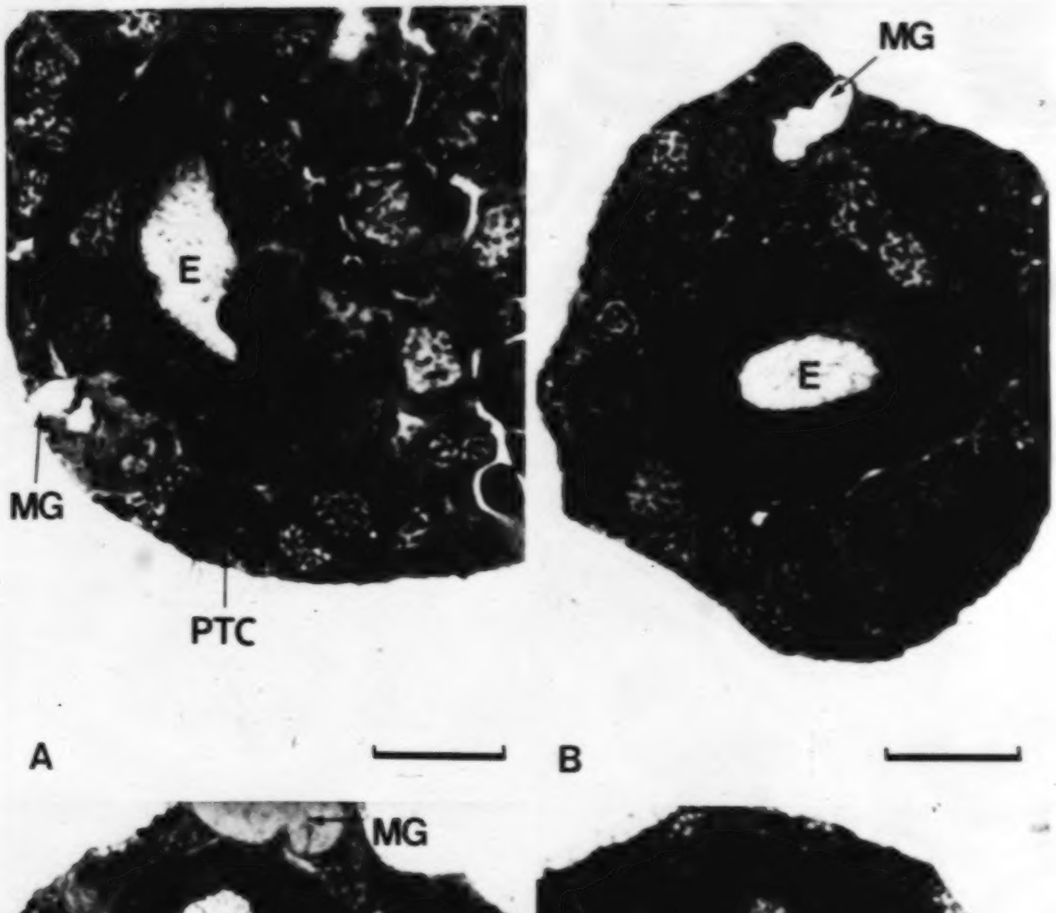

E
MG
PTC
A
MG
E
B
MG
E
SS
ST
C
E
MG
C
ST
D

Figure 37: Development of gland cells, II.

A. Frontal section of a 12 day trochophore showing the lateral and apical mucoid glands. Scale bar = 5 µm

B. Frontal section of a 12 day trochophore showing the presence of mucoid glands in the elongating hyposphere. Scale bar = 5 µm

C. Sagittal section of a metatrochophore showing the appearance of loculated gland cells in the episphere. Note that the mucoid gland cells are the major cell type of the episphere. Scale bar = 10 µm

D. Sagittal section of a metatrochophore showing the mucoid and loculated gland cells of the pygidium. Note the presence of large lipid droplets within the stomach cells. Scale bar = 10 µm

Legend:

E - Esophagus
L - Lipid droplet
I - Intestine
LG - Loculated gland cell
MG - Mucoid gland
SS - Setal sac
ST - Stomach

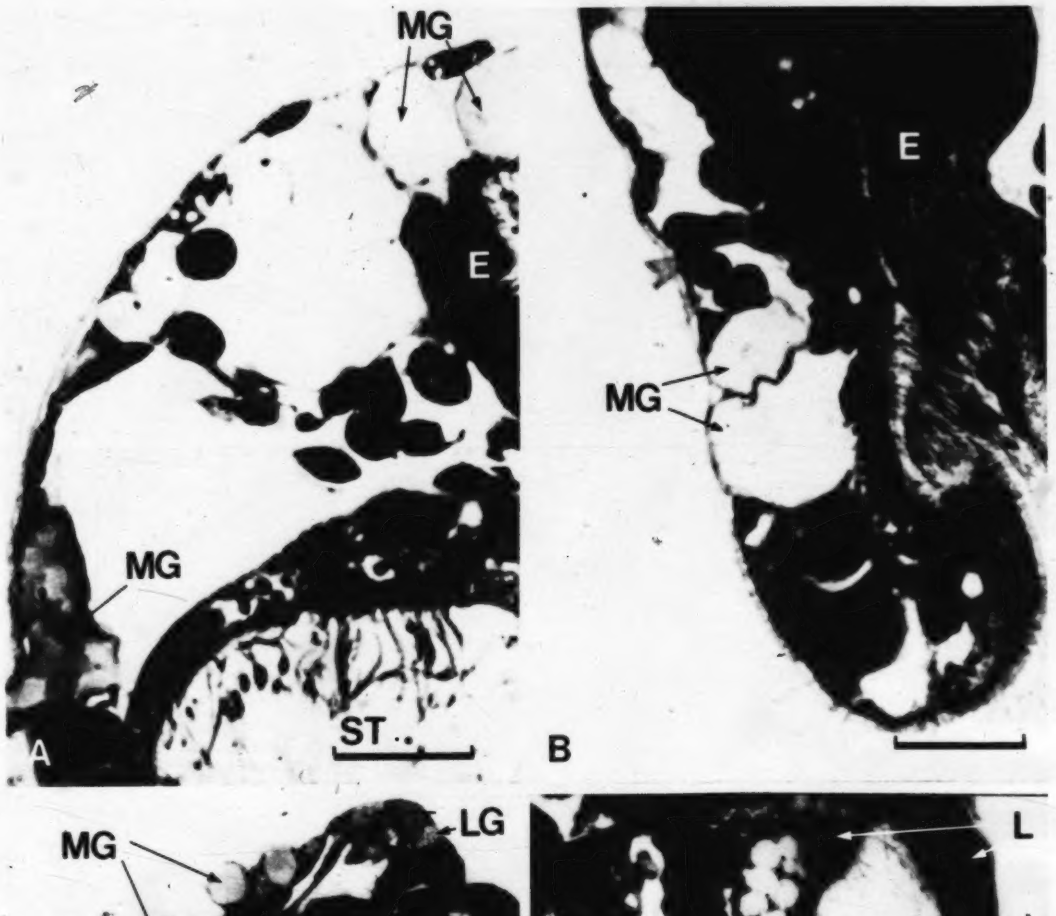

MG
E
MG
ST
A
E
MG
B
MG
LG
SS
C
L
ST
LG
MG
D

Figure 38: Development of gland cells, III.

A. Sagittal section of a competent larva showing the mucoid gland cells located in the episphere and pygidium. Scale bar = 20 µm

B. Sagittal section of a competent larva showing gland cell types A, B, C, and E. Note the large setal sac. Scale bar = 10 µm

C. Sagittal section of a competent larva showing gland cell types C and D. Note the building organ located below the oral lip fold and prominent longitudinal muscle. Scale bar = 10 µm

D. Cross-section of a competent larva showing the building organ composed of type A and B gland cells. Note the prominent gland cells located within the posterior region of the esophagus. Scale bar = 10 µm

Legend:

BO - Building organ
EG - Esophageal glands
gA - Gland cell type A
gB - Gland cell type B
gC - Gland cell type C
gD - Gland cell type D
gE - Gland cell type E
LIP- Oral lip fold
LM - Longitudinal muscle
MG - Mucoid gland cells
SS - Setal sac
VC - Vacuolated cells

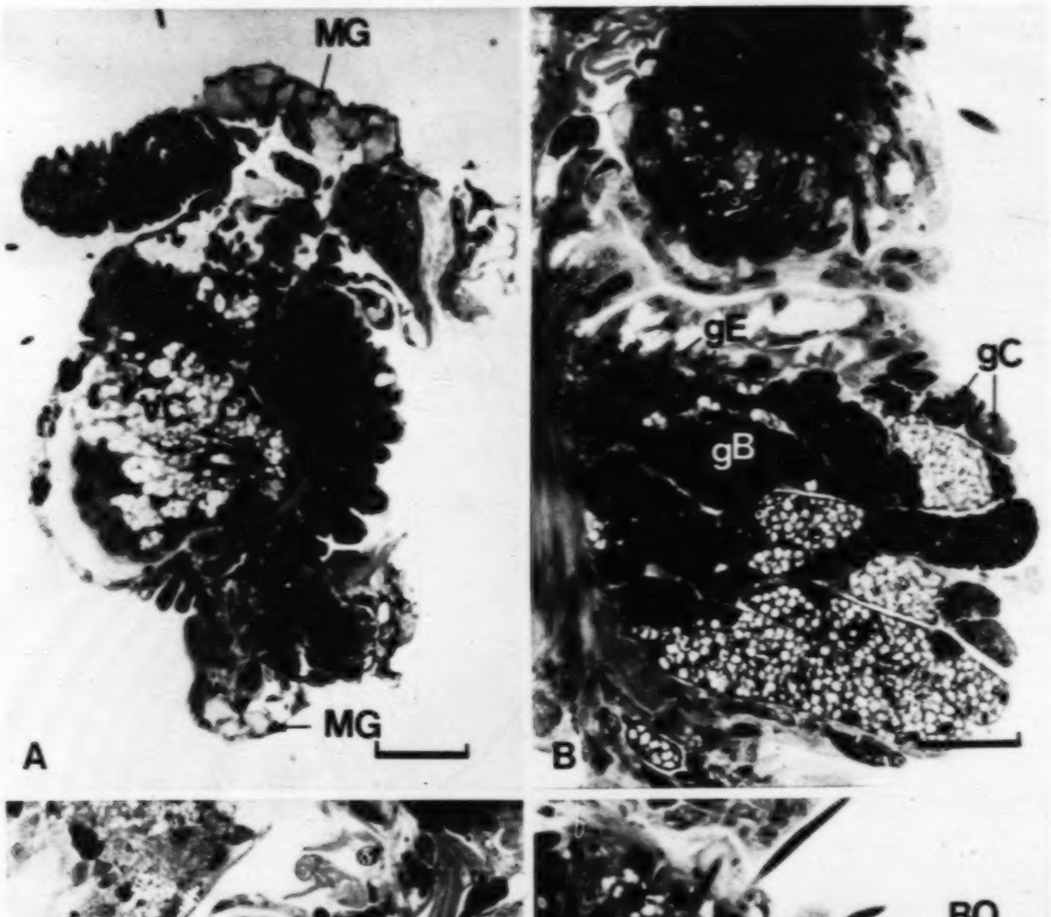

MG
VC
MG
A
gE
gC
gB
B
LIP
BO
gD
gC
C
BO
D

Figure 39: TEM of apical gland cells in a 5 day trochophore.

A. Frontal section through the apical unicellular mucoid gland showing the fibrillar material comprising the mucoid secretion. Scale bar = 1 µm

B. Enlargement of mucoid gland. Arrows indicate the thin cell membranes surrounding the mucoid secretion. Scale bar = 1 µm

Legend:

CU - Cuticle
E - Esophagus
G - Gland
N - Nucleus

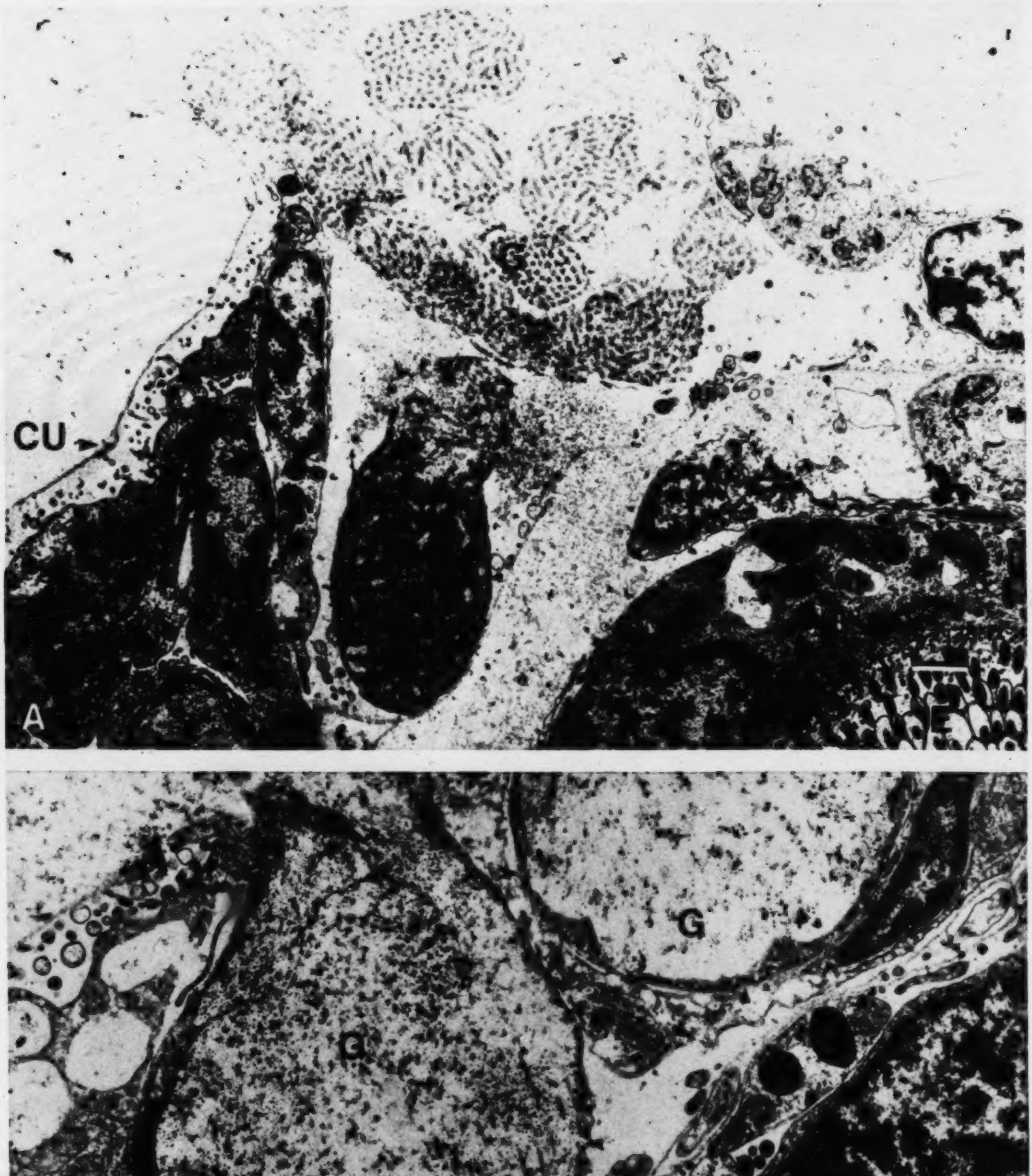

CU
G
E
A
G
G
B

Figure 40: Larval tentacles.

A. Frontal section of a metatrochophore with tentacle buds. Note the ring muscle of the tentacle connecting to the supraesophageal muscle and to the peritoneum of the longitudinal muscle. Situated within the peritoneum of the longitudinal muscle is a circumesophageal blood vessel. Scale bar = 10 µm

B. Cross-section through the distal ends of the tentacles of a competent larva showing type D and E gland cells. Note the sensory tuft. Scale bar = 10 µm

C. Longitudinal section of a tentacle of a competent larva showing the longitudinal muscles. Scale bar = 10 µm

D. Cross-section through the proximal end of a tentacle showing the lateral and medial coelomic cavities which are separated by a blood vessel. Note the insertion of the longitudinal muscles onto the ring muscles. Scale bar = 5 µm

Legend:

BV - Blood vessel
CEV - Circumesophageal blood vessel
CT - Ciliary tract
E - Esophagus
gD - Gland cell type D
gE - Gland cell type E
LC - Lateral coelomic cavity
LM - Longitudinal muscle
MC - Medial coelomic cavity
P - Peritoneum
RM - Ring muscle
SEM - Supraesophageal muscle
SEV - Supraesophageal blood vessel
ST - Sensory tuft
T - Tentacle

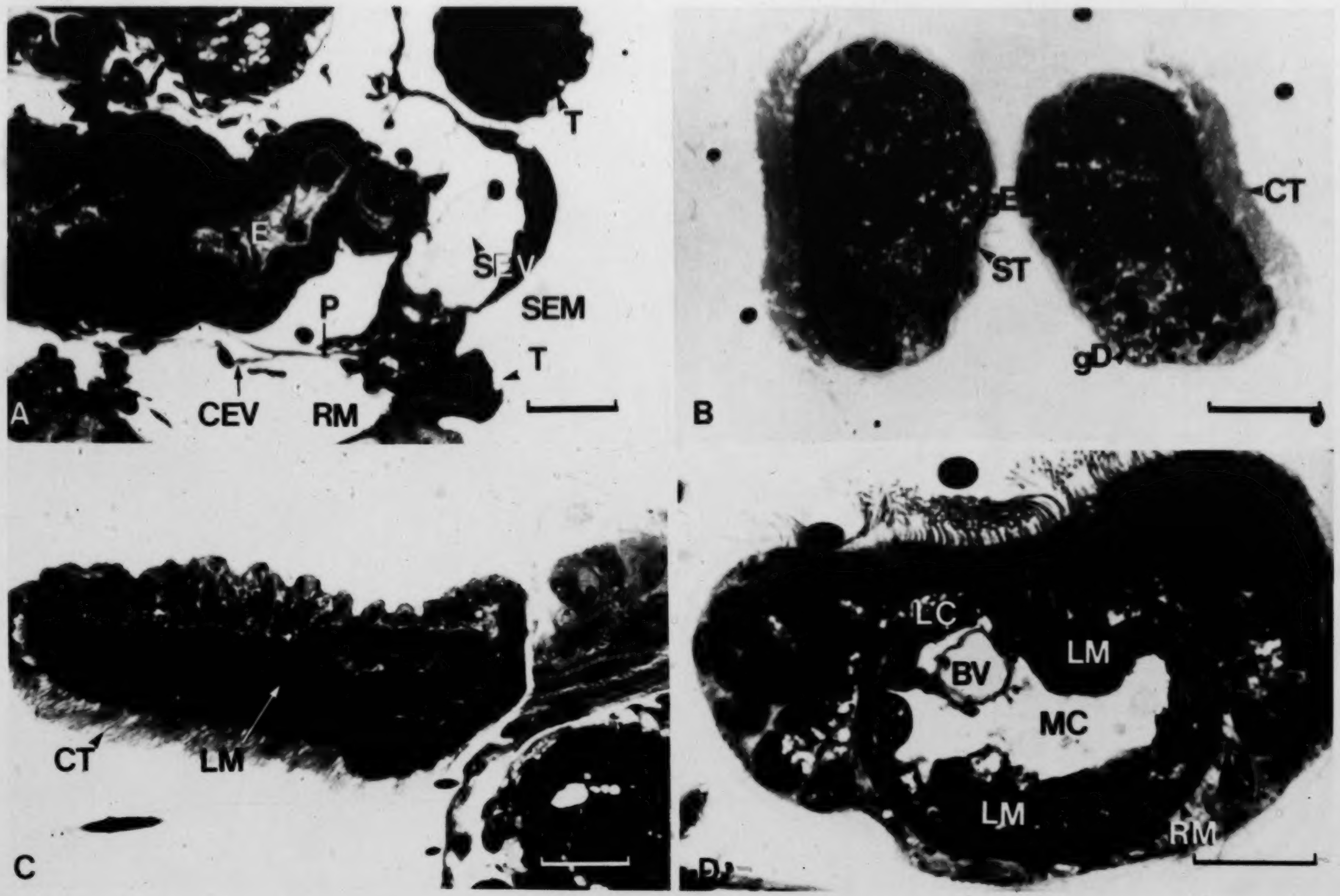

T
E
SEV
SEM
P
T
A
CEV
RM
E
CT
ST
gD
B
CT
LM
C
LC
BV
LM
MC
LM
RM
D

Figure 41: Diagrammatical representation of gregarious settlement of the larvae of *S. cementarium* in the dish containing the tube sand of *Idanthrysus ornamentatus*.
Scale bar = 300 µm

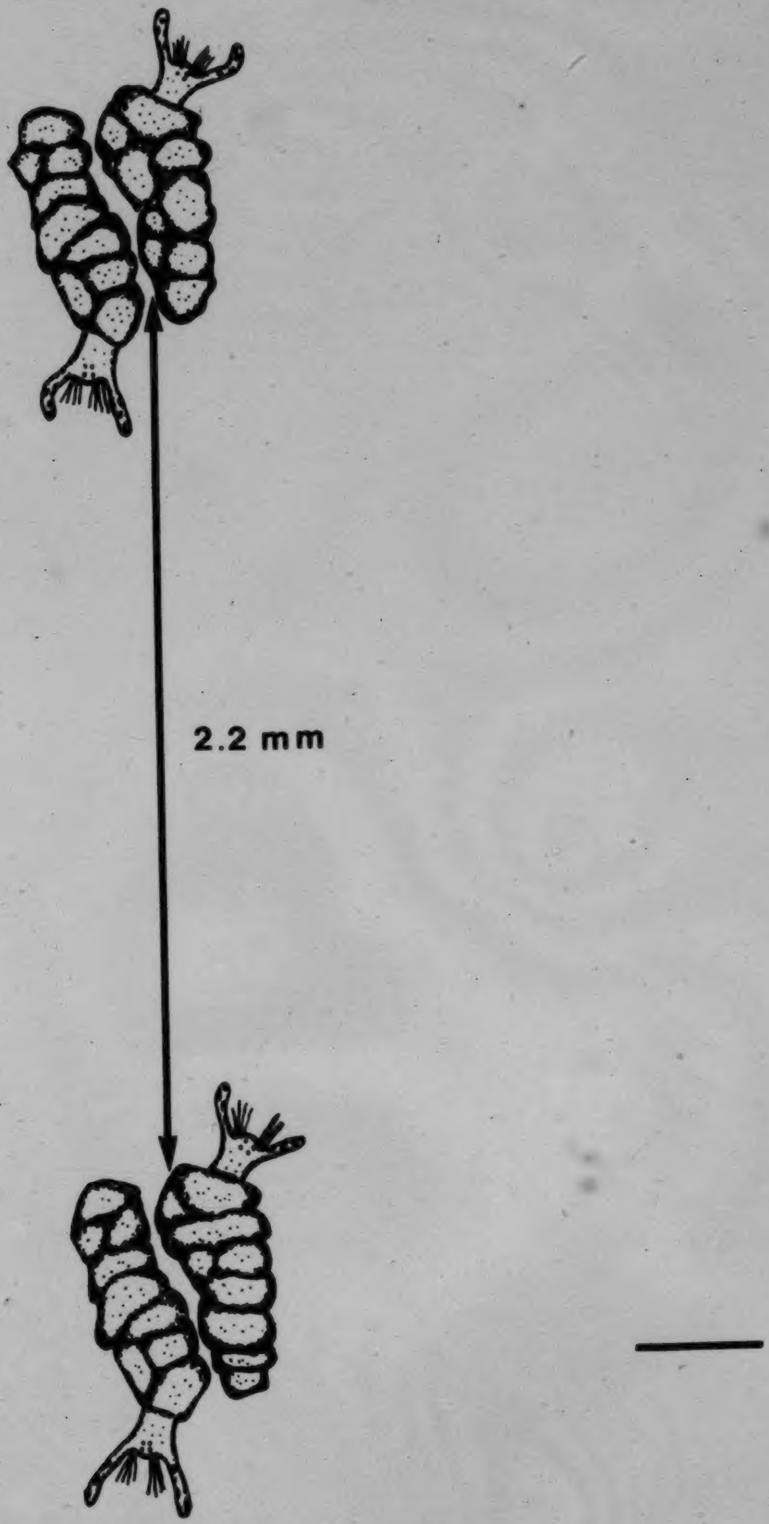

2.2 mm

Figure 42: Diagrammatical representation of gregarious settlement of the larvae of *S. cementarium* in the dish containing the tube sand of *Phragmatopoma lapidosa*.
Scale bar = 300 µm

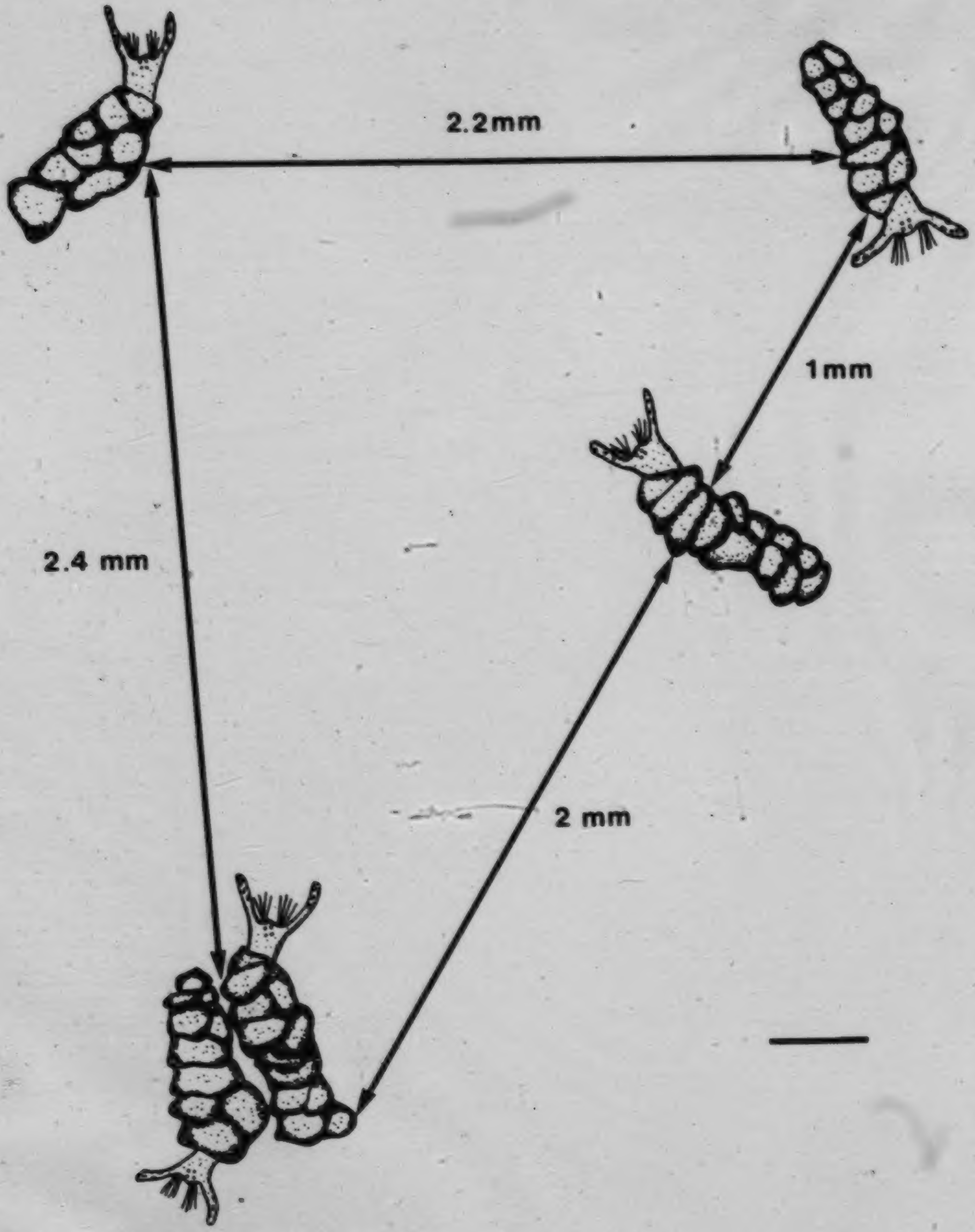

2.2mm
1mm
2.4 mm
2 mm

Figure 43: Metamorphic changes in the cuticle and the coelomic cavities.

A. Sagittal section of a 2 day post-settled juvenile showing the parathoracic cuticle in the region of glandular discharge. Note the presence of gland cell types A and B. Scale bar = 10 µm

B. Sagittal section of a 3 day post-settled juvenile showing the bilayered cuticle. Scale bar = 10 µm

C. Cross-section of a 1 day post-settled juvenile showing the newly formed head coelom. Note that the primary coelom is retained.
Scale bar = 10 µm

D. Sagittal section of a 3 day post-settled juvenile showing the pygidial coelomic cavity. Note the numerous loculated gland cells within the pygidium and the reduction in number of mucoid gland cells.
Scale bar = 10 µm

Legend:

CU - Cuticle
gB - Gland cell type B
HC - Head coelom
LG - Loculated gland cells
LM - Longitudinal muscles
MG - Mucoid gland cells
PC - Primary coelomic cavity
PS - Parathoracic setal sac
PYC - Pygidial coelomic cavity
SS - Setal sac
ST - stomach

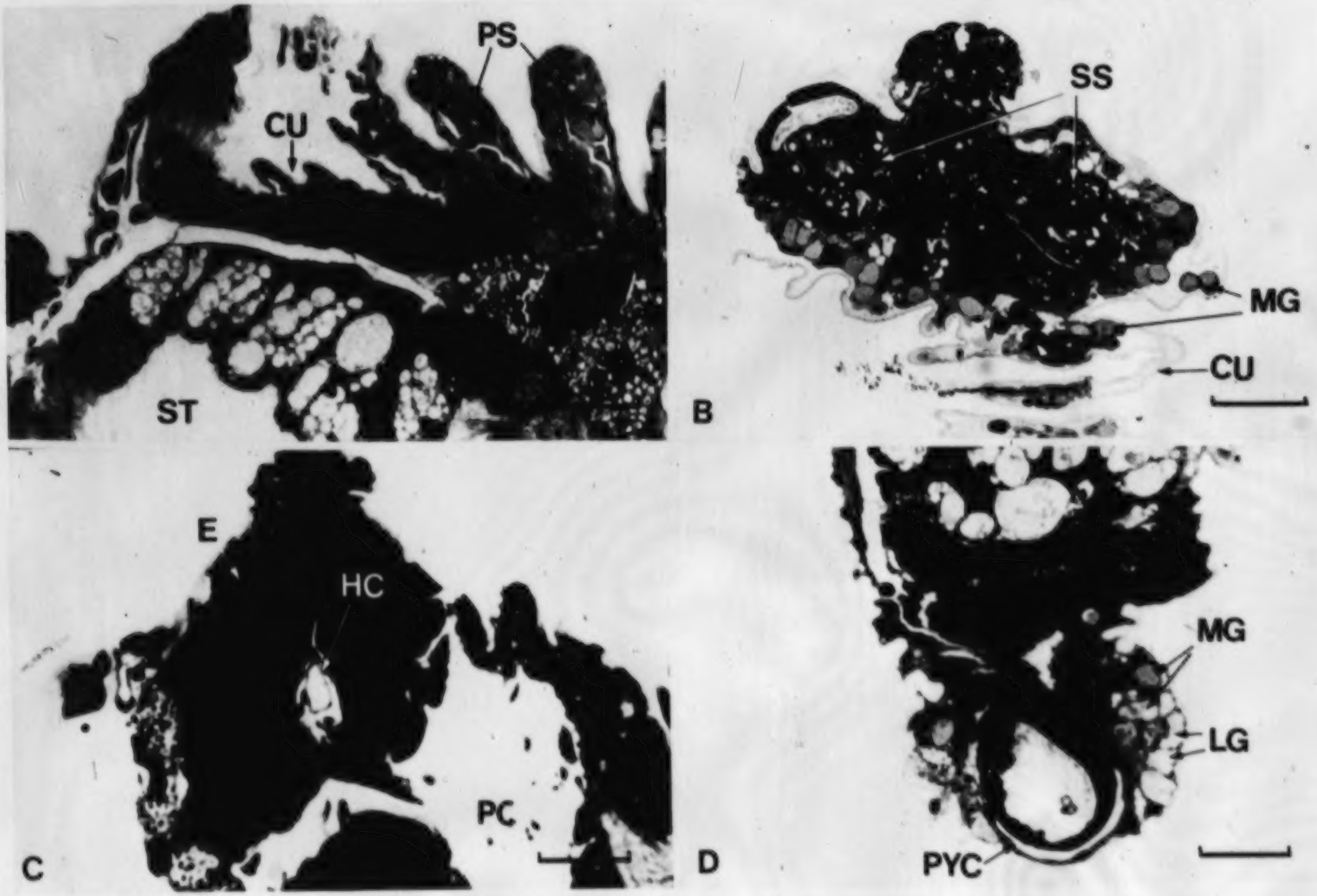

PS
CU
ST
B
SS
MG
CU
E
HC
PC
C
D
MG
LG
PYC

Figure 44: Metamorphic changes in the epidermis.

A. Cross-section of 1 day post-settled juvenile showing the prototrochal cell. Note the reduction in the number of prototrochal cilia. Scale bar = 5 µm

B. Sagittal section through the prototrochal region of a 2 day post-settled juvenile. The prototrochal cell is barely visible due to the atrophy of the cell. Note the settling paleae in the setal sac. Scale bar = 5 µm

C. Sagittal section through the pygidium of a 3 day post-settled juvenile showing the reduction in the size of the telotrochal cell and the number of cilia. Scale bar = 5 µm

D. Sagittal section of a 3 day post-settled juvenile showing the numerous mucoid glands in the epidermis. The prototrochal cell is no longer visible. Scale bar = 5 µm

Legend:

LG - Loculated gland cell
MG - Mucoid gland cell
P - Settling paleae
PT - Prototrochal cell
SS - Setal sac
TT - Telotrochal cell

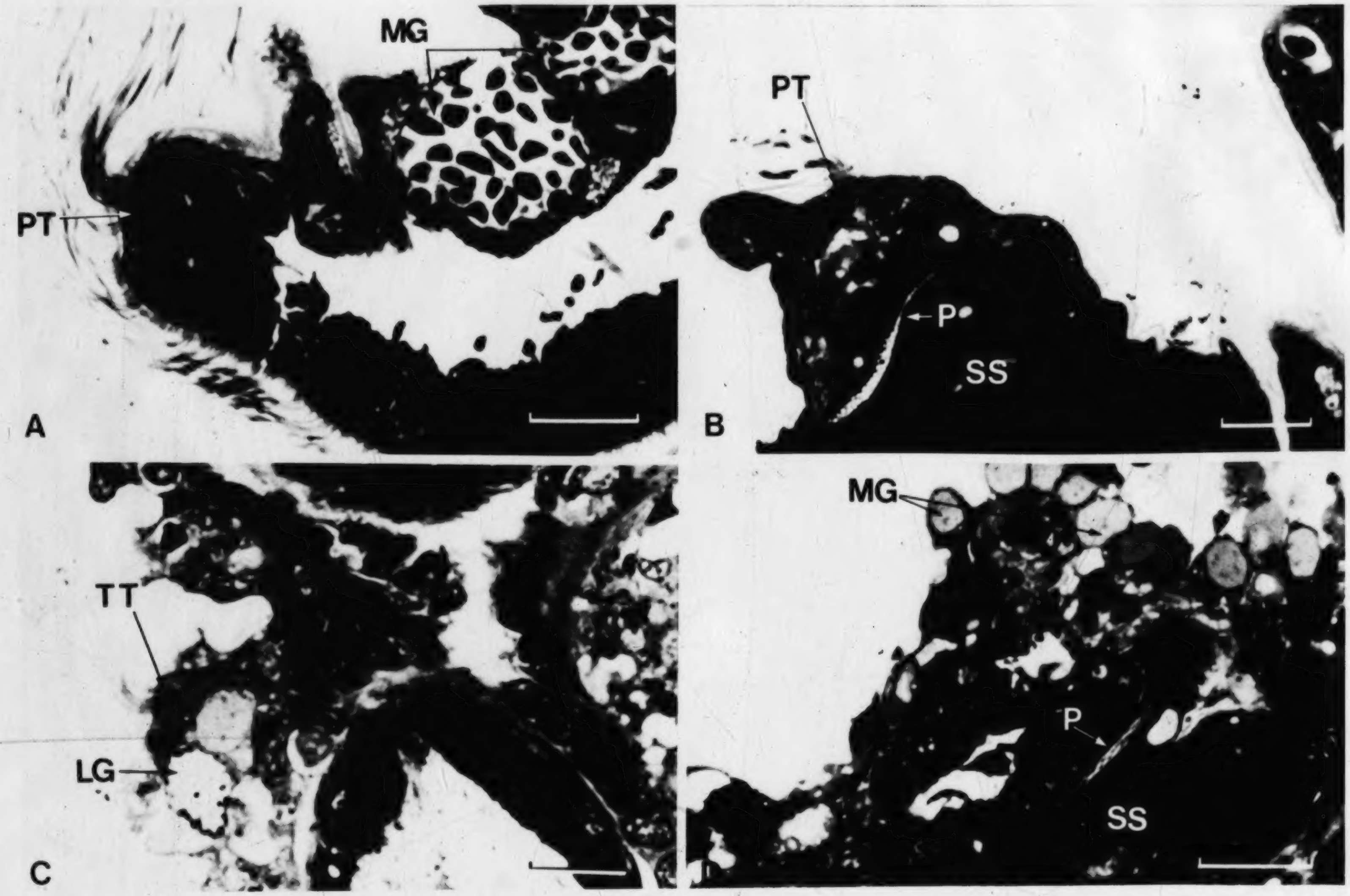

MG
PT
A
PT
P
SS
B
TT
LG
C
MG
P
SS

Figure 45: Metamorphic changes in the setal sacs.

A. Cross-section of a 1 day post-settled juvenile showing the setal sacs containing the settling paleae. Note that the setal sacs are unchanged. Scale bar = 10 µm

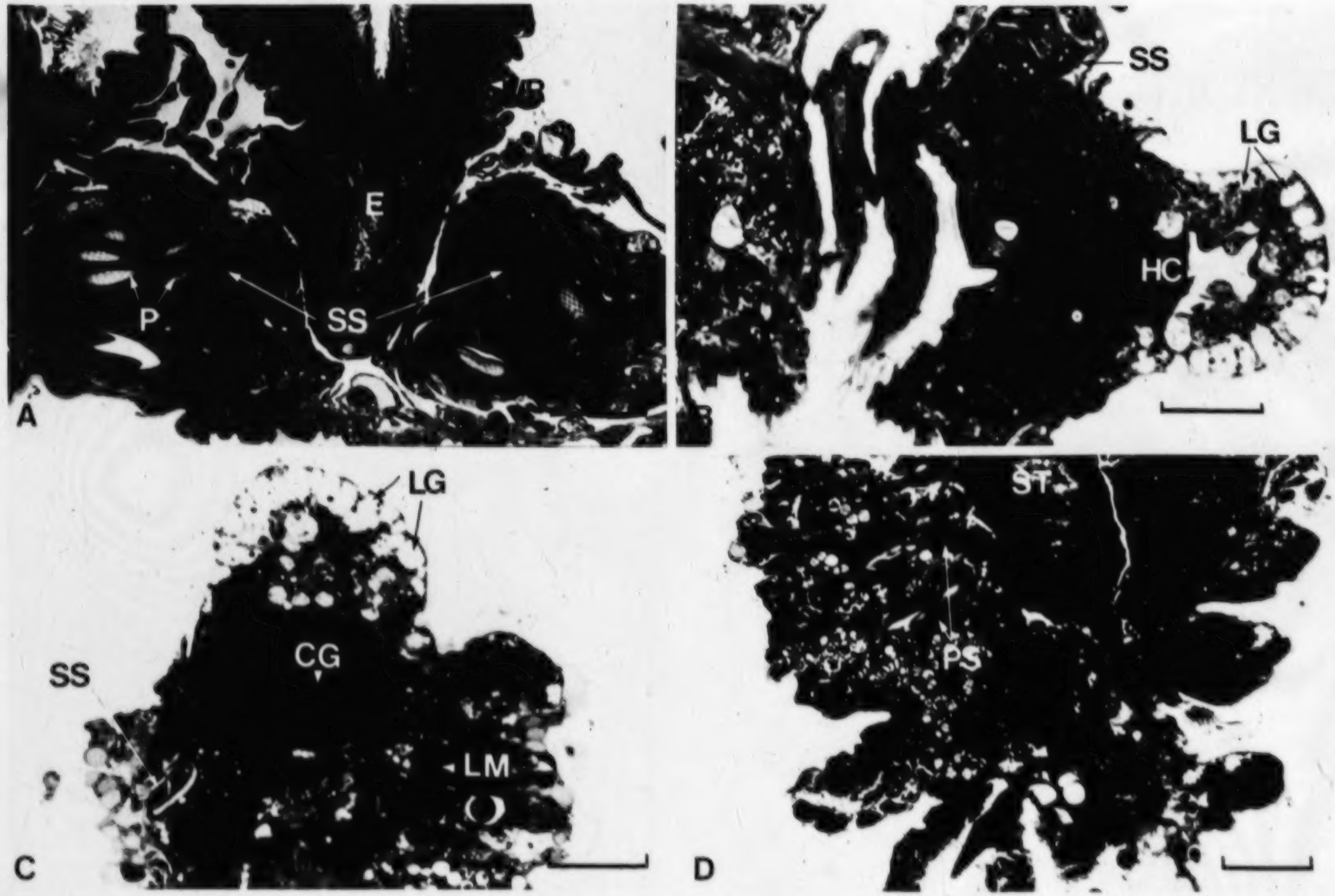
B. Sagittal section of a 2 day post-settled juvenile showing the reduction in size of the setal sacs, presumably due to histolysis. Note the loculated gland cells in the prostomium and the head coelom. Scale bar = 10 µm

C. Sagittal section of a 3 day post-settled juvenile showing the further reduction of the setal sacs. Scale bar = 10 µm

D. Sagittal section of a 3 day post-settled juvenile showing the setal sacs of the parathoracic parapodia. Note that they have remained unchanged during metamorphosis. Scale bar = 10 µm

Legend:

CG - Cerebral ganglion
E - Esophagus
HC - Head coelom
LG - Loculated gland cells
LM - Longitudinal muscles
P - Settling paleae
PS - Parathoracic setal sacs
SS - Setal sac
ST - Stomach



E
P
SS
A
SS
LG
HC
LG
CG
SS
LM
C
ST
PS
D

Figure 46: Metamorphic changes in the muscle system, I.

A. Cross-section of a 1 day post-settled juvenile showing the disorganization of the medial branch of the prototrochal muscle. At this time the setal sac muscles resemble those of the competent larva. Scale bar = 10 µm

B. Cross-section of a 1 day post-settled juvenile showing the broken band of longitudinal muscles. Scale bar = 10 µm

C. Cross-section of a 1 day post-settled juvenile showing the broken connections of the supraesophageal muscle and the setal sac muscles to the longitudinal muscles. Arrows indicate the broken muscle bundles. Scale bar = 10 µm

D. Cross-section of a 1 day post-settled juvenile showing the dorsal and ventral longitudinal muscles which remain unchanged during metamorphosis. Scale bar = 10 µm

Legend:

DLM - Dorsal longitudinal muscle
E - Esophagus
I - Intestine
LM - Longitudinal muscle
MBPTM - Medial branch of the prototrochal muscle
SEM - Supraesophageal muscle
SSM - Setal sac muscle
VLM - Ventral longitudinal muscle

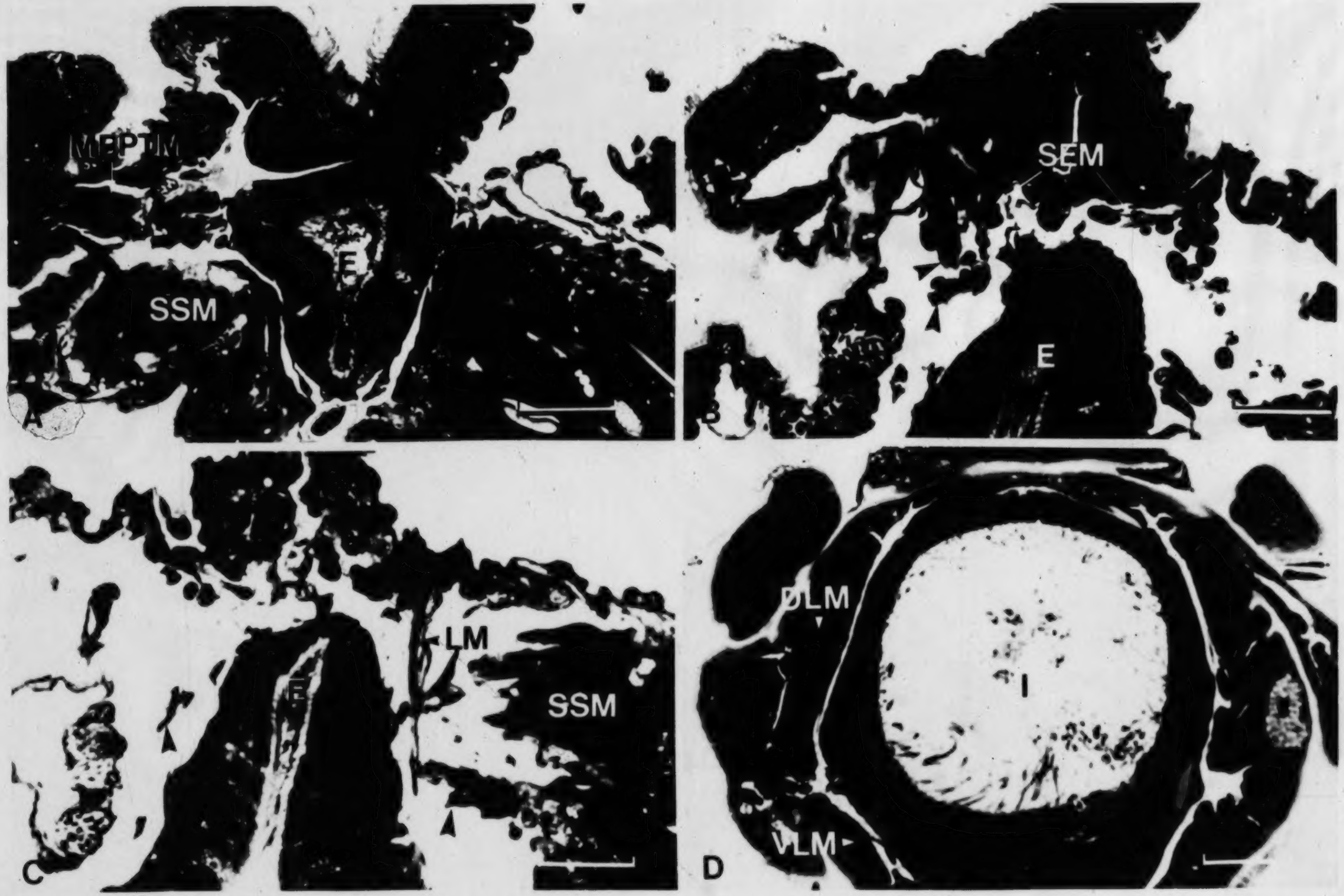

MBPTM
SSM
E
A
SEM
E
B
LM
SSM
E
C
DLM
I
VLM
D

Figure 47: Metamorphic changes in the muscle system, II.

A. Sagittal section of a juvenile 2 days post-settlement. Arrows indicate the broken connections of the supraesophageal muscle to the longitudinal muscle. Scale bar = 10 µm

B. Sagittal section of a juvenile 2 days post-settlement showing the thin posterior connection of the setal sac muscles to the longitudinal muscles. Scale bar = 10 µm

C. Sagittal section of a juvenile 3 days post-settlement showing the longitudinal muscles. Scale bar = 10 µm

D. Sagittal section of a 3 days post-settled juvenile showing the setal sac muscle which connects to the longitudinal muscles. Scale bar = 10 µm

Legend:

CG - Cerebral ganglion
E - Esophagus
EY - Eyespot
gA - Gland cell type A
gB - Gland cell type B
LM - Longitudinal muscles
SS - Setal sac
SSM - Setal sac muscles
ST - Stomach
T - Tentacle

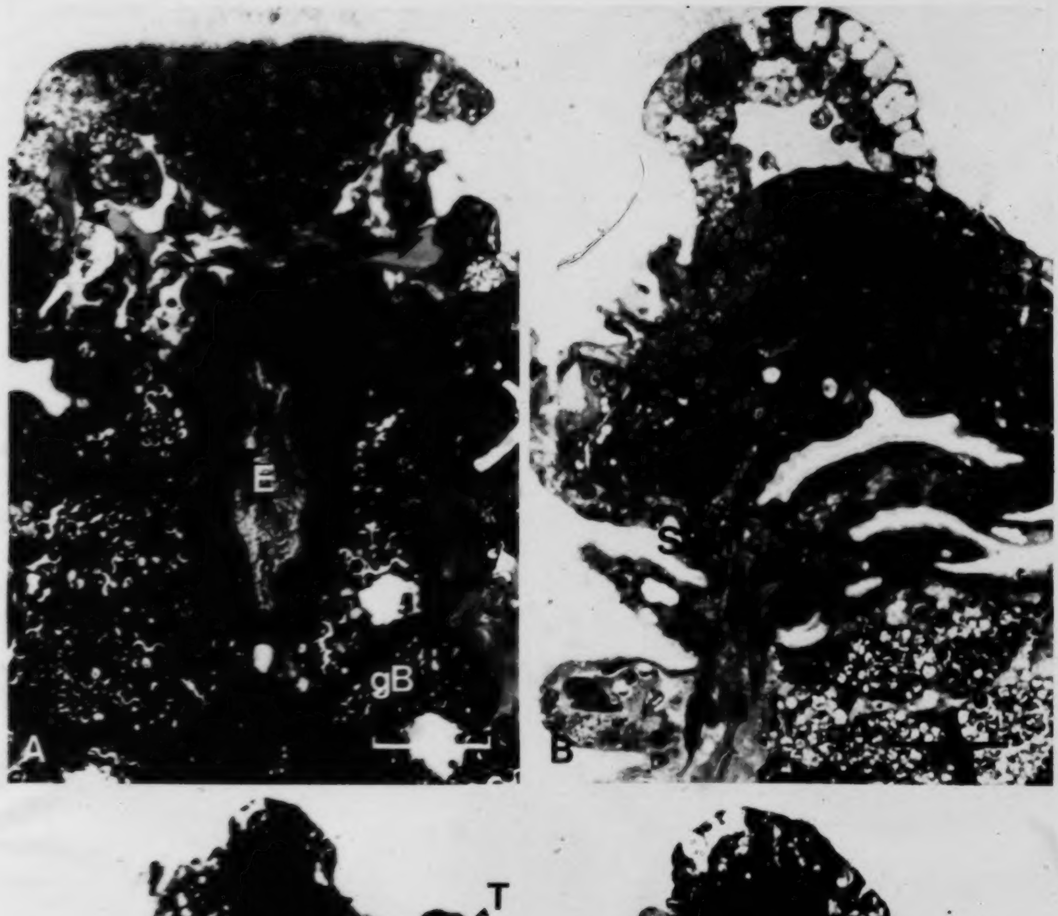

E
gB
A
S
B
T
LM
C
EY
SS
SSM
MG
LM
gA

Figure 48: Metamorphic changes in the alimentary tract, I.

A. Cross-section through the esophagus of a 1 day post-settled juvenile showing the light and dark staining esophageal cells and the esophageal glands. Note the absence of the esophageal glands of the competent larva which contained the flocculent material. Scale bar = 10 µm

B. Cross-section through the stomach of a 1 day post-settled juvenile showing the vacuolated cells which have not yet undergone hypertrophy. Scale bar = 10 µm

C. Sagittal section of a juvenile 2 days post-settlement showing the absence of gland cells in the esophagus. Scale bar = 10 µm

D. Sagittal section of a juvenile 3 days post-settlement showing the esophagus. Note that the dark-staining cells are now scarce. Scale bar = 10 µm

Legend:

BO - Building organ
DBS - Dorsal blood sinus
DC - Dark-staining esophageal cells
E - Esophagus
EG - Esophageal glands
gA - Gland cell type A
gB - Gland cell type B
LC - Light-staining esophageal cells
PT - Prototrochal cell
SS - Setal sac
ST - Stomach
VC - Vacuolated cells

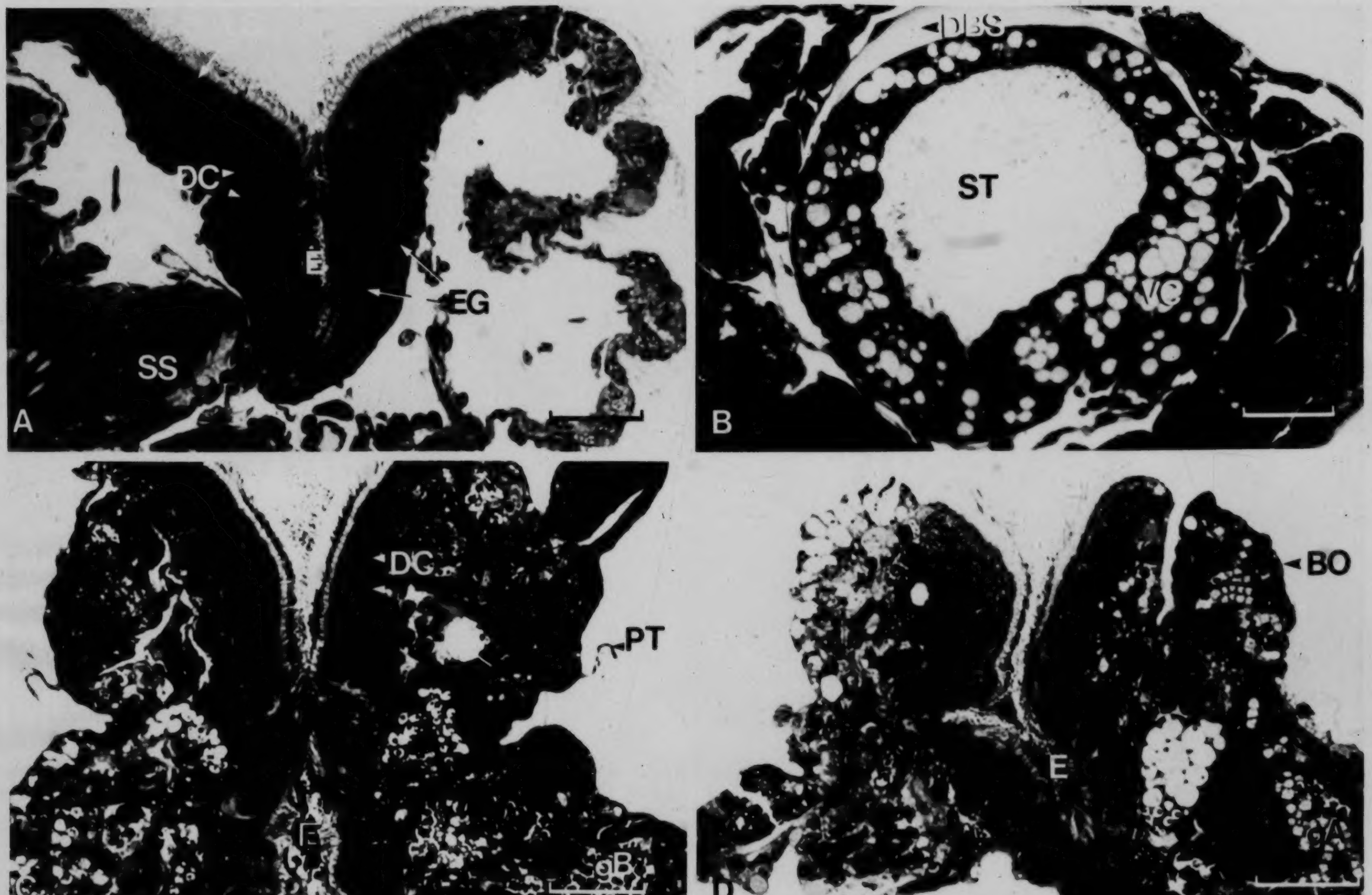

DC
E
EG
SS
A
DBS
ST
VC
B
DC
PT
E
gB
C
BO
E
gA
D

Figure 49: Metamorphic changes in the alimentary tract, II.

A. Sagittal section of a juvenile 2 days post-settlement showing the hypertrophy of the vacuolated cells of the upper stomach. Scale bar = 10 µm

B. Sagittal section of a juvenile 3 days post-settlement showing the continued hypertrophy of the vacuolated cells and their dissociation. Note the cellular debris within the intestine, indicated by the arrows. Scale bar = 10 µm

C. Enlargement of the cellular debris within the intestine showing the dissociated vacuolated cells and the presumptive zymogen granules. Scale bar = 5 µm

D. Sagittal section of a juvenile 3 days post-settlement showing the esophagus. Note the epidermal mucoid droplet in the esophagus and the obliteration of the esophageal-stomach junction by the hypertrophy of the vacuolated cells. Scale bar = 10 µm

Legend:

E - Esophagus
LM - Longitudinal muscles
MD - Mucoid droplet
PZG - Presumptive zymogen granules
S - Septum
SS - Setal sac
ST - Stomach
VC - Vacuolated cells

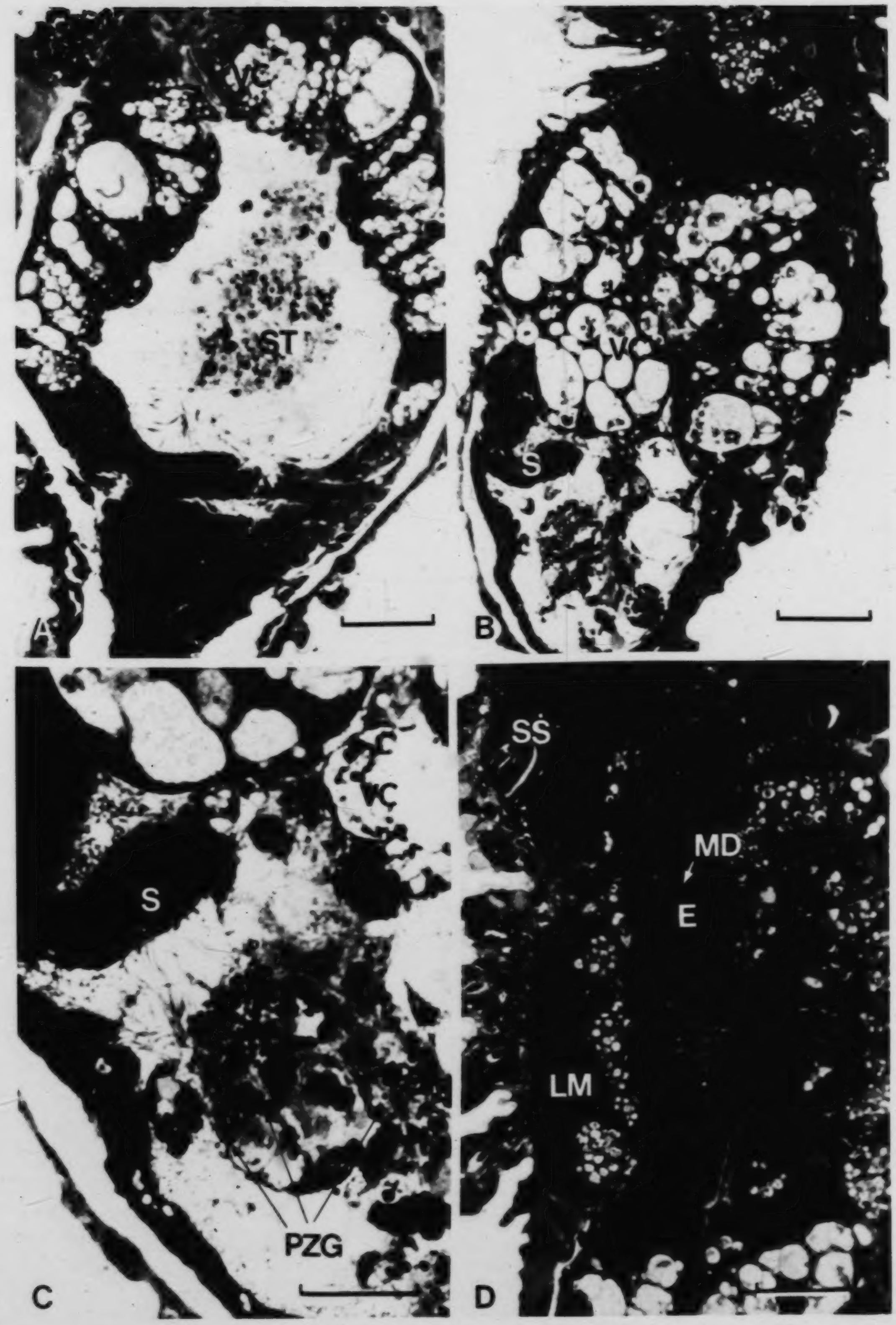

VC
ST
A
B
V
S
C
S
VC
PZG
D
SS
MD
E
LM

Figure 50: Metamorphic changes in the nervous system.

A. Cross-section through the prostomium of a juvenile 1 day post-settlement showing the optic ganglia and well defined eyespots. Note the enlargement of the cerebral ganglion. Scale bar = 10 µm

B. Sagittal section of a 3 day post-settled juvenile showing the shortening of the ventral root of the circumesophageal commissure. Note the epidermal mucoid droplet within the esophagus. Scale bar = 10 µm

C. Cross-section of a 1 day post-settled juvenile showing the subesophageal ganglion located above the building organ. Note the glandularity of the building organ. Scale bar = 10 µm

D. Cross-section of a 1 day post-settled juvenile showing the paired ventral nerve cords. Scale bar = 10 µm

Legend:

BO - Building organ
CG - Cerebral ganglion
CEC - Ventral root of the circumesophageal commissure
DBS - Dorsal blood sinus
E - Esophagus
EY - Eyespot
LM - Longitudinal muscles
MD - Mucoid droplet
PS - Parathoracic setal sac
SEG - Subesophageal ganglion
ST - Stomach
VNC - Ventral nerve cord

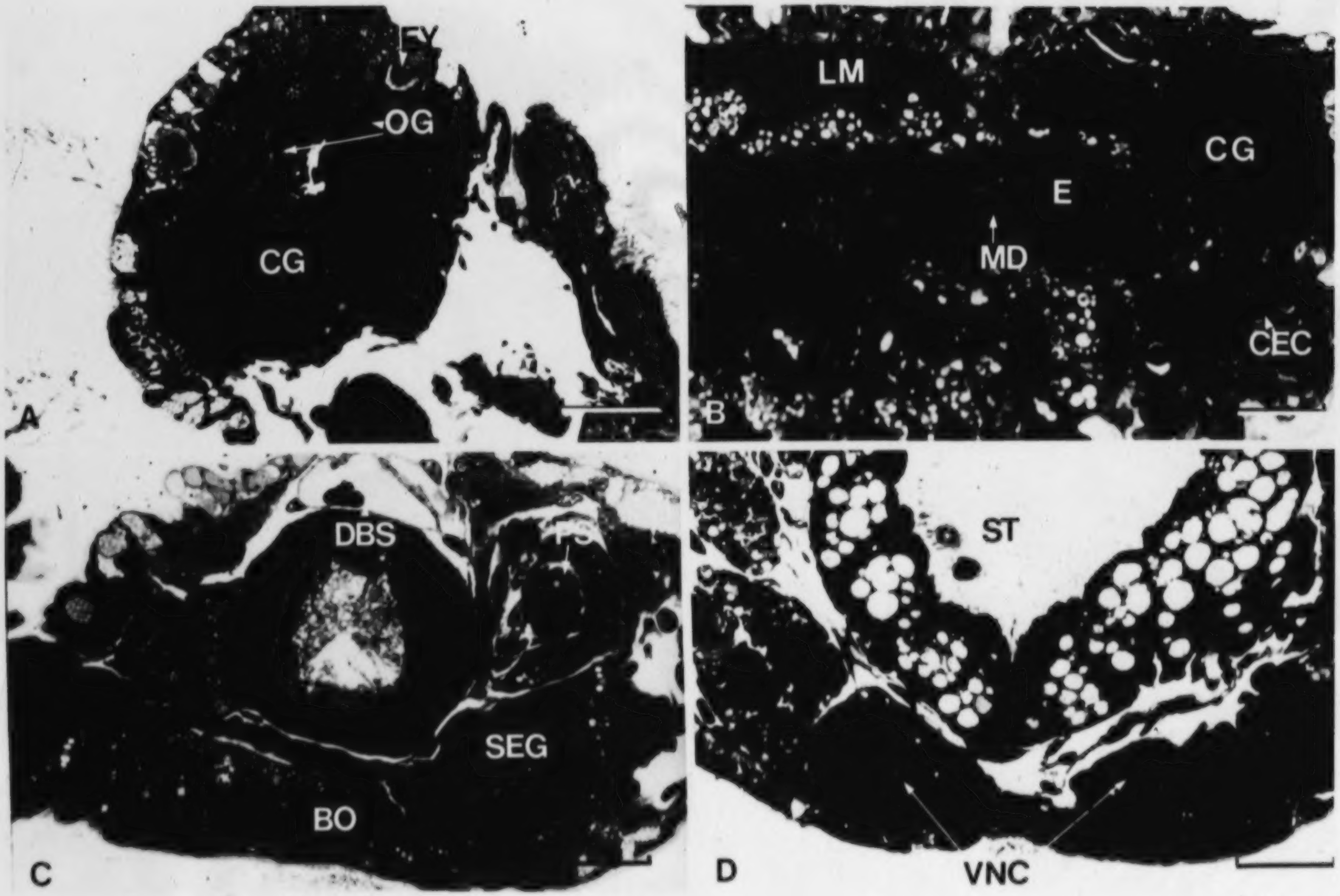

EY
OG
CG
A
LM
CG
E
MD
CEC
B
DBS
PS
SEG
BO
C
ST
VNC
D

Figure 51: Metamorphic changes in the gland cells.

A. Cross-section of the prostomium of a juvenile one day post-settlement showing the internal reorganization of the lateral mucoid gland cells. Note the numerous loculated gland cells which have appeared in the prostomium. Scale bar = 10 µm

B. Cross-section of the pygidium of a juvenile one day post-settlement showing the reorganization of the mucoid gland cells. Note the presence of the loculated gland cells. Scale bar = 10 µm

C. Sagittal section of a 2 day post-settled juvenile. Note the reduction in number of type B gland cells and the absence of type A gland cells. Scale bar = 20 µm

D. Sagittal section of a 3 day post-settled juvenile showing the reduction in the number of type A gland cells. Note the absence of type B gland cells and the glandularity of the building organ. Scale bar = 20 µm

Legend:

BO - Building organ
E - Esophagus
EM - Eyespot
gA - Gland cell type A
gB - Gland cell type B
gC - Gland cell type C
LG - Loculated glands
LM - Longitudinal muscles
MG - Mucoid gland cells
SEG - Subesophageal ganglion
ST - Stomach
TT - Telotroch
UL - Uncinigerous lobe
VNC - Ventral nerve cord

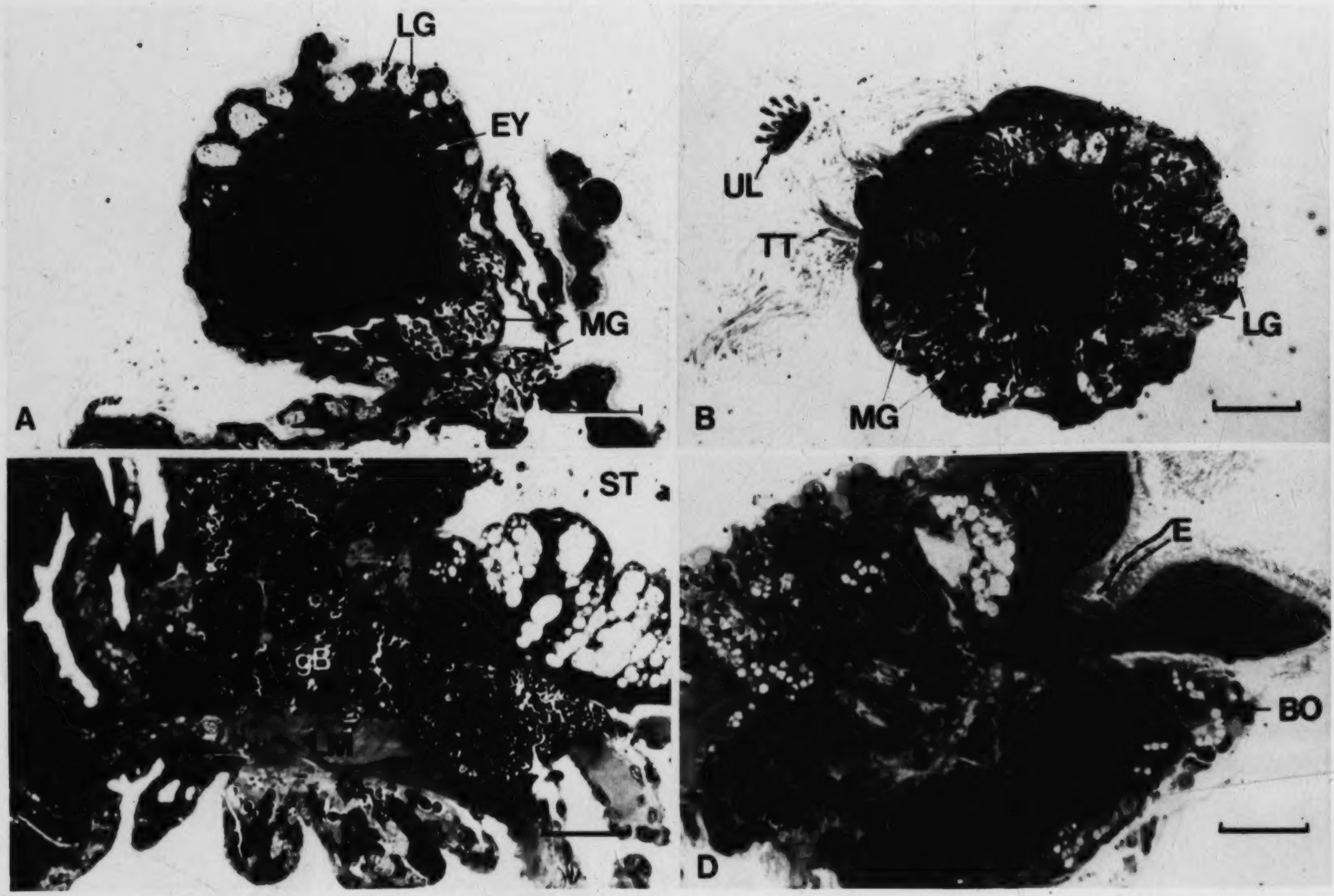

LG
EY
MG
A
UL
TT
MG
LG
B
ST
gB
LM
E
BO
D

Figure 52: Micrographs of the adult.

A. Cross-section of the adult epidermis showing the bilayered cuticle and the epidermal mucoid glands. Scale bar = 10 µm

B. Cross-section showing the dorsal blood vessel and stomach. Note the acellular blood plasma in the blood vessel and the absence of vacuolated cells in the stomach. Scale bar = 20 µm

C. Cross-section showing the circular muscle below the epidermis and the longitudinal muscles. Note the ventral nerve cord. Scale bar = 20 µm

D. Cross-section showing one-half of the building organ. Note the glandularity of it. Scale bar = 20 µm

Legend:

BO - Building organ
BV - Blood vessel
C - Coelom
CM - Circular muscle
CU - Cuticle
LM - Longitudinal muscles
MG - Mucoid gland
ST - Stomach

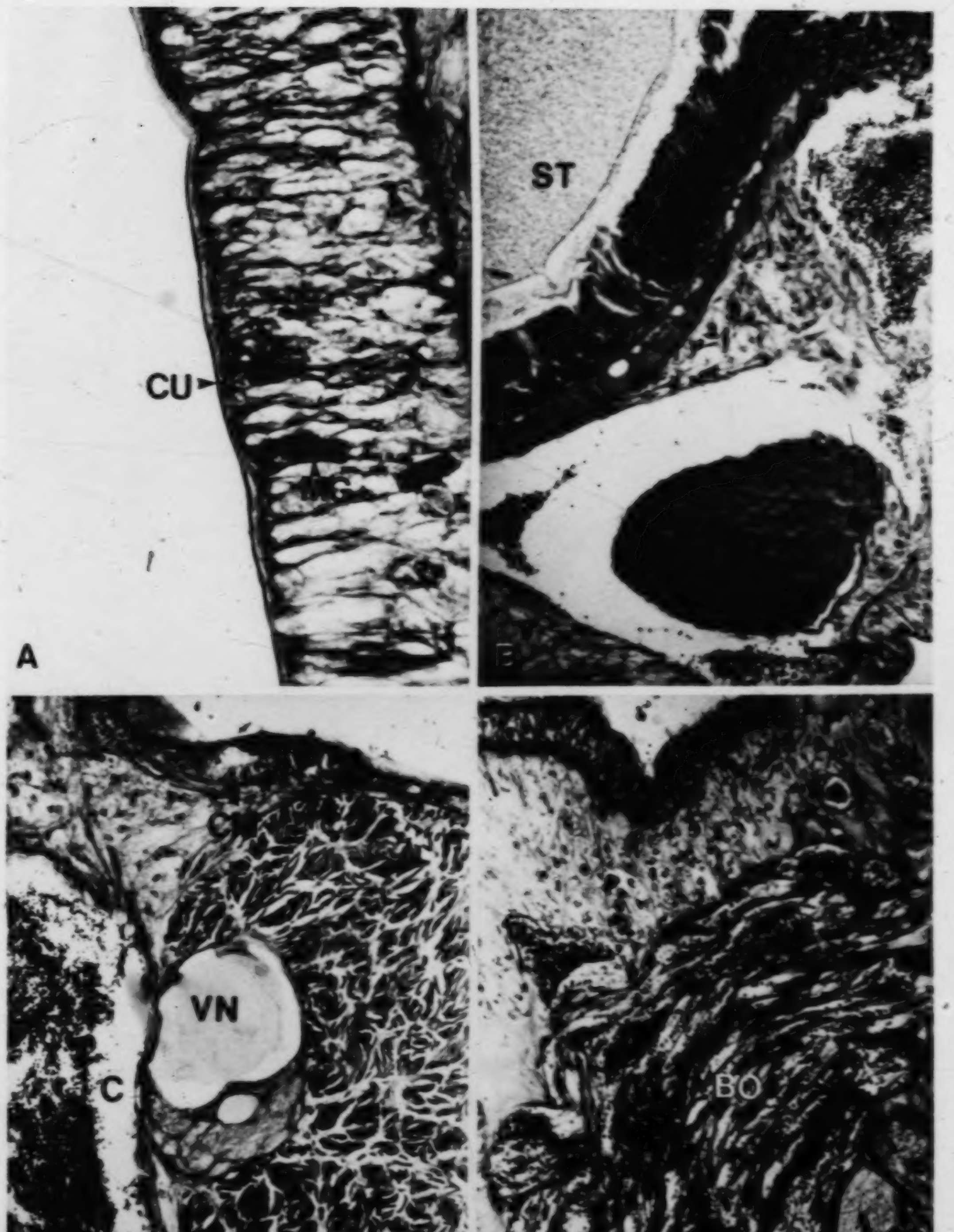

CU
A
ST
B
VN
C
C
BO
D

Figure 53: Juvenile stages of S. cementarium.

A. Ventral view of newly metamorphosed juvenile. Note the anterior rotation of the tentacles and enlarged building organ. Scale bar = 80 µm

B. Ventral view of the prostomium of the juvenile figured in A, showing the presence of the settling paleae within the setal sacs and loss of the provisional setae. Scale bar = 80 µm

C. Ventral view of juvenile 5 days after settlement. The forming caudal appendage can be seen below the pygidial pigmentation. Scale bar = 80 µm

D. Dorsal view of juvenile 18 days after settlement. Note the primary tentacles and the two shorter pairs as well as the now prominent caudal appendage. The settling paleae have been replaced by opercular paleae. Scale bar = 160 µm

Legend:

BO - Building organ
CA - Caudal appendage
OC - Opercular cirri
OP - Operculum
T - Tentacle
UL - Uncinigerous lobe

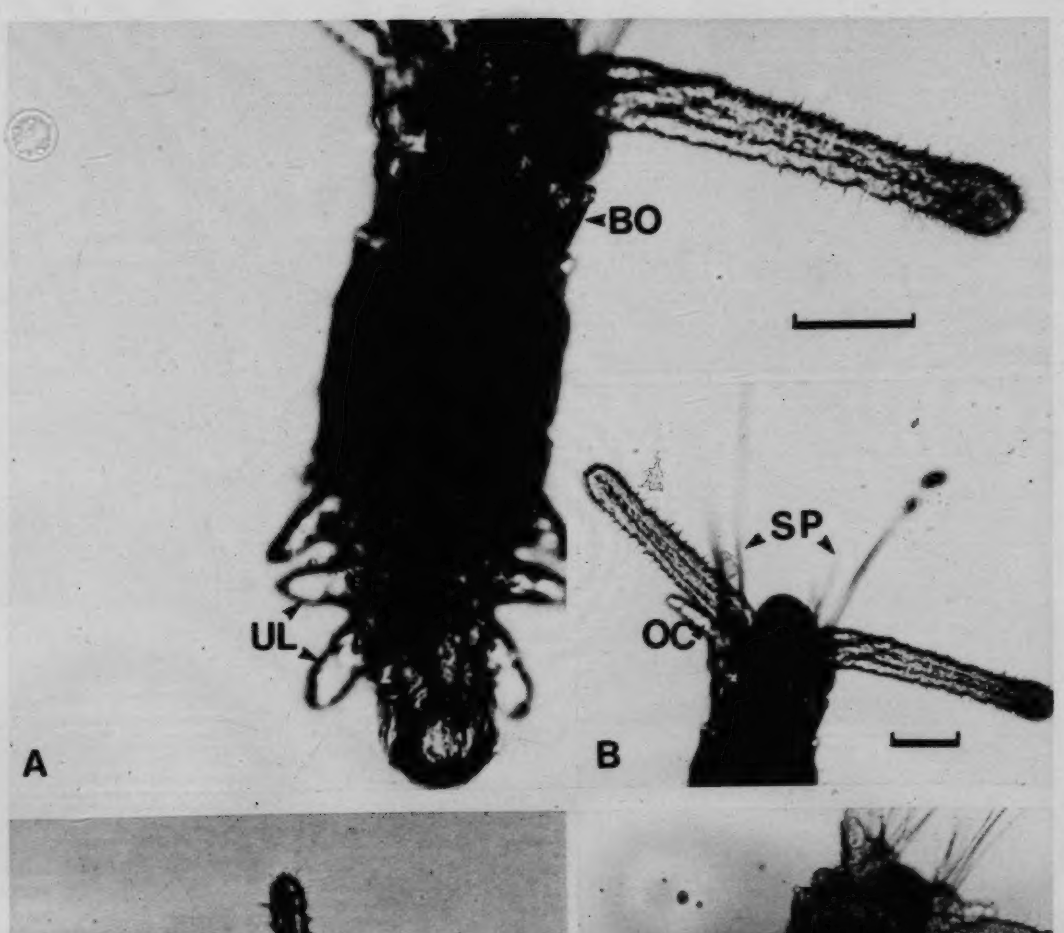

BO
UL
A
SP
OC
B
T
C
CA
OP
T
CA
D

Figure 54: SEM of juvenile worm 18 days after settlement.

A. Dorsal view of juvenile. Note that there are still only 3 parathoracic and 3 abdominal segments present. Scale bar = 50 µm

B. Lateral view of the tentacle of the juvenile showing the ciliary tract on the ventral surface and the ciliary tufts in the regions of the corrugations on the tentacles. Scale bar = 5 µm

C. Enlargement of the dorsal prostomial and thoracic regions showing the prominent ciliary sensory tufts located in these regions. Scale bar = 20 µm

Legend:

A - Abdomen
CG - Ciliary groove
CT - Ciliary tuft
OP - Operculum
PT - Parathorax
ST - Sensory tuft
T - Tentacle

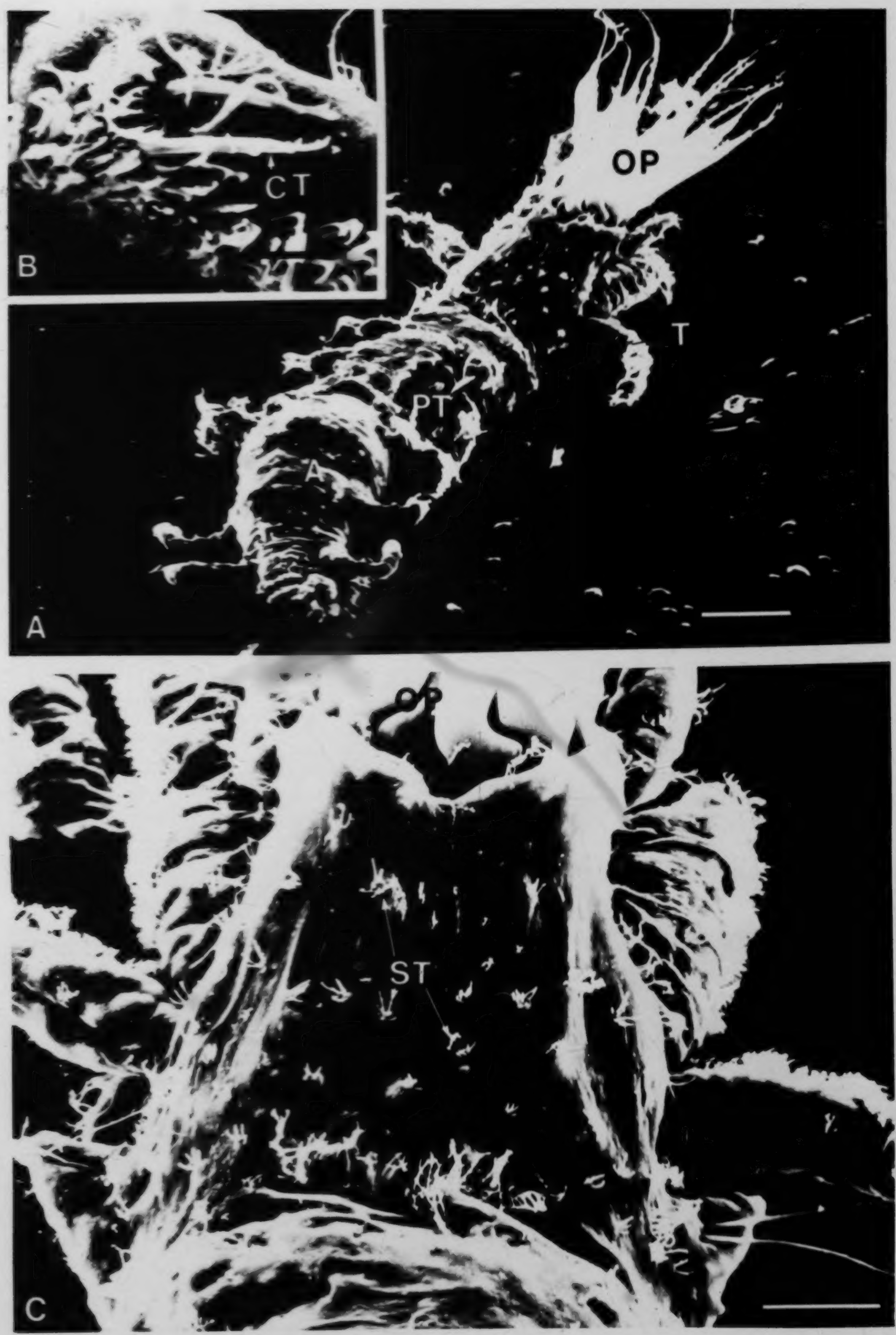

CT
B
OP
T
PT
A
A
ST
C

Figure 55: SEM of a juvenile 18 days after settlement showing the abdomen and operculum.

A. Right, lateral view of the abdomen of the juvenile showing the caudal appendage and the large uncinigerous lobes. Scale bar = 25 µm

B. Frontal view of the operculum. Note that some settling paleae are still present amongst the primary opercular paleae of the juvenile's operculum. Scale bar = 20 µm

Legend:

CA - Caudal appendage
POP - Primary opercular paleae
SP - Settling paleae
UL - Uncinigerous lobe

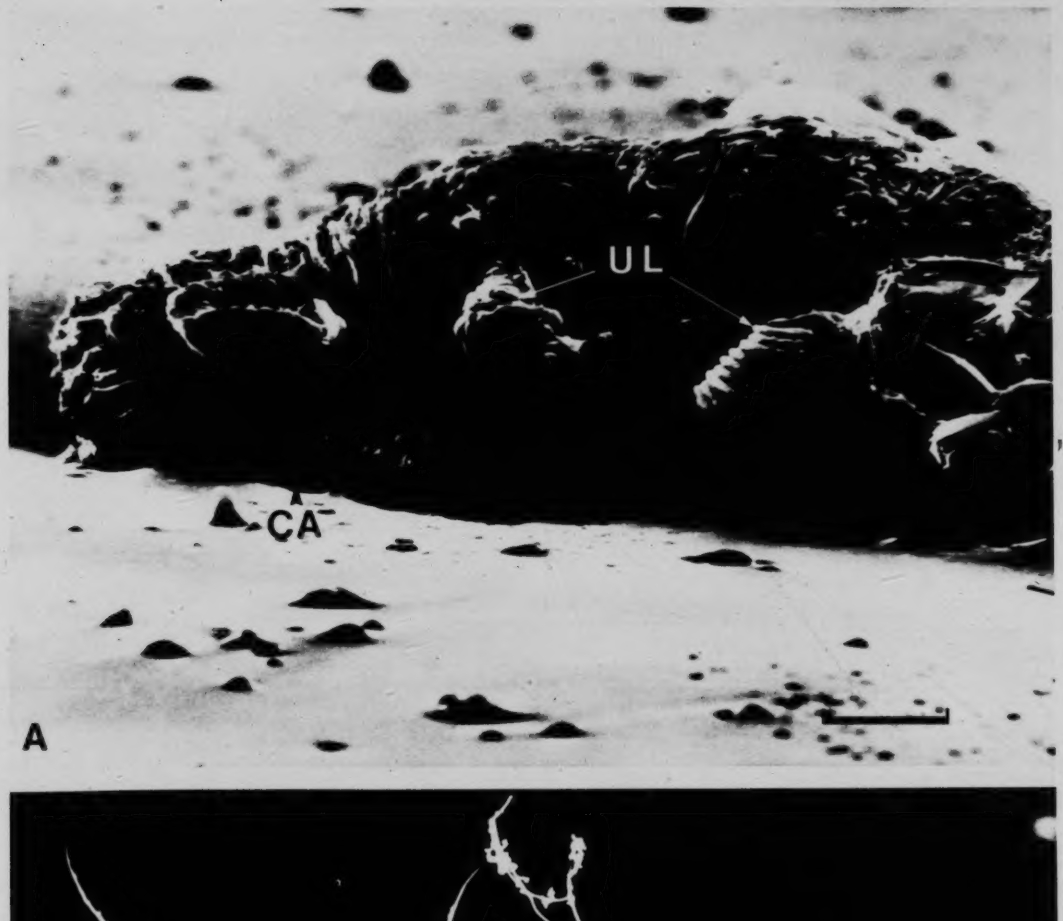

UL
CA
A
POP
SP
B

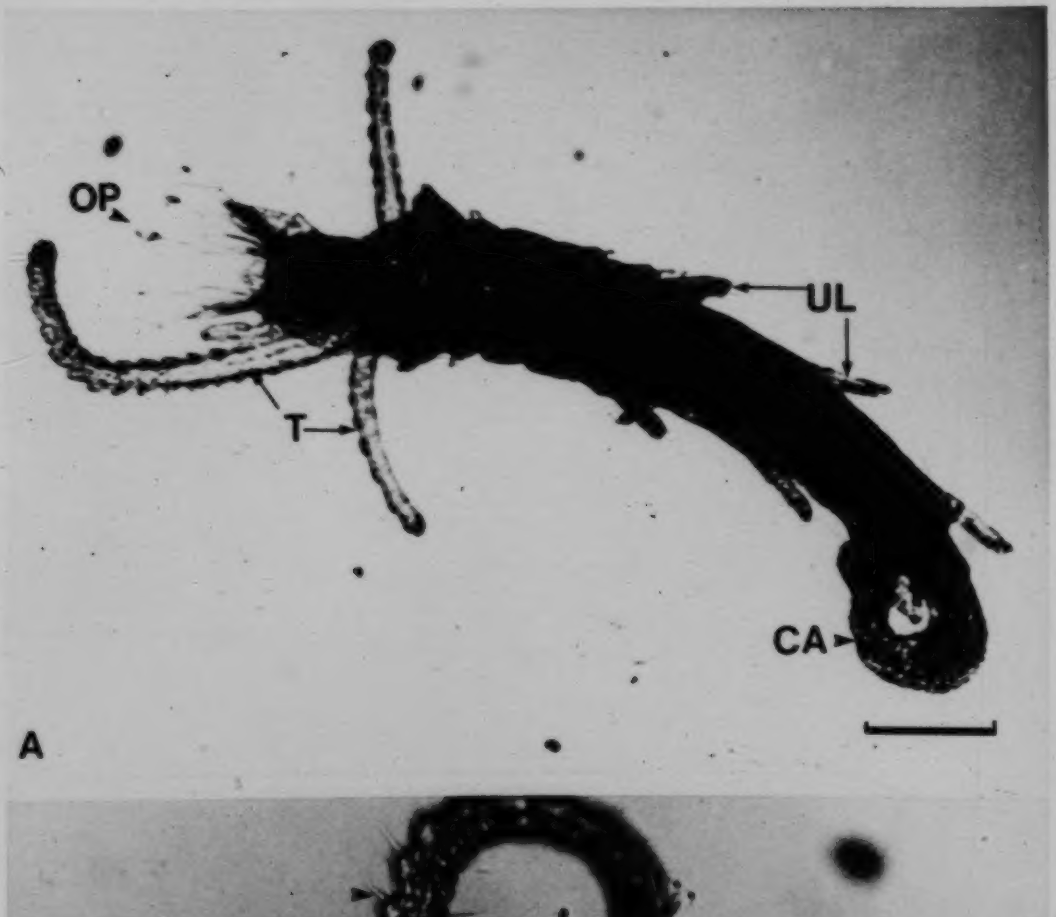
Figure 56: Juvenile S. cementarium, 38 days post-settlement

A. Ventral view of juvenile. Note the elongation of the abdominal segments and caudal appendage compared to the 18 day post-settlement juvenile in Figure 53D. Scale bar = 180 µm

B. Enlargement of prostomium of juvenile figured in A showing the outer and inner paleae comprising the operculum. Arrows indicate the thick cilia found at the corrugations on the tentacles. Scale bar = 40 µm

Legend:

CA - Caudal appendage
CG - Ciliary groove
IOP - Inner opercular paleae
OOP - Outer opercular paleae
OP - Operculum
T - Tentacle
UL - Uncinigerous lobe


OP
UL
T
CA
A
OOP
OP
CG
B

# LITERATURE CITED

Åkesson, B. 1961. On the histological differentiation of the larvae of Pisione remota. Acta zool. (Stockh.) 42: 117-225.

Åkesson, B. 1962. The embryology of Tomopteris helgolandica (Polychaeta). Acta zool. (Stockh.) 43: 135-199.

Åkesson, B. 1967a. The embryology of the polychaete Eunice kobiensis. Acta. zool. (Stockh.) 48: 141-192.

Åkesson, B. 1967b. On the nervous system of the Lopadorhynchus larva (Polychaeta). Ark. Zool. 20: 55-78.

Åkesson, B. 1968. The ontogeny of the glycerid prostomium. Acta. zool. (Stockh.) 49: 203-217.

Allen, M.J. 1959. Embryological development of the polychaetous annelid Diopatra cuprea (Bosc). Biol. Bull. 116: 339-361.

Allen, M.J. 1964. Embryological development of a syllid Autolytus fasciatus (Bosc) (Class Polychaeta). Biol. Bull. 127: 187-205.

Anderson, D.T. 1959. The embryology of the polychaete Scoloplos armiger. Quart. J. micr. Sci. 100: 89-166.

Anderson, D.T. 1966. The comparative embryology of the Polychaeta. Acta zool. (Stockh.) 47: 1-42.

Anderson, D.T. 1973. Embryology and Phylogeny in Annelids and Arthropods. Pergamon Press. New York.

Barnes, R.D. 1980. Invertebrate Zoology. Saunders College/Holt, Rinehart and Winston. Philadelphia.

Bass, N.G., G. Chapman, and J.M. Chapman. 1969. Uptake of leucine by larvae and adults of *Nereis*. Nature 221: 476-477.

Bhaud, M. 1969. Développement larvaire de *Phalacrostemma cidariophilum* Marenzeller, 1895. Vie et Milieu, ser. A. 20: 543-558.

Bhaud, M. 1972. Quelques données sur le déterminisme écologique de la reproduction des annélides polychètes. Mar. Biol. 17: 115-136.

Bhaud, M. 1975. Nouvelles données sur les larves de Sabellariidae récoltées en Méditerranée. Ann. Inst. océanogr. 51: 155-172.

Bickell, L.R. 1978. Larval development, metamorphosis, and juvenile feeding of *Doridella steinbergae* (Lance) (Opisthobranchia: Nudibranchia). M.Sc. Thesis, University of Alberta.

Bickell, L.R., F.S. Chia, and B.J. Crawford. 1981. Morphogenesis of the digestive system during metamorphosis of the nudibranch *Doridella steinbergae* (Gastropoda): Conversion from phytoplanktivore to carnivore. Mar. Biol. 62: 1-16.

Blake, J.A. 1969. Reproduction and larval development of *Polydora* from northern New England.

(Polychaeta: Spionidae). Ophelia 7: 1-63.

Bobin, G. and H. Mazoué. 1944. Topographie, histologie, caractères physiques et chimiques d' *Aphrodite aculeata* L. (Annélide Polychète). Bull. Soc. Zool. France 69 : 125-134.

Bouligand, Y. 1966. Etude ultrastructurale des soies et des cellules associées chez une annélide polychète *Haplosyllis depressa*. C.R. Acad. Sci. (Paris) 263D: 378-381.

Bouligand, Y. 1967. Les soies et les cellules associées chez deux annélides polychètes. Etude en microscopie photonique à contraste de phase et en microscopie électronique. Z. Zellforsch. mikrosk. Anat. 79: 332-363.

Brandenburg, J. 1970. Die cuticula des *Dinophilus* (Archiannelida). Z. Morphol. Tiere 68: 300-307.

Briggs, J.C. 1974. Marine Zoogeography. McGraw Hill, Inc. New York.

Brökelmann, J. and A. Fischer. 1966. Über die cuticula von *Platynereis dumerilli* (Polychaeta). Z. Zellforsch. mikrosk. Anat. 70: 131-135.

Bubko, O.V., Y.S. Minichev, T.G. Lvova, and E.E. Kulakovsky. 1979. The development of the nervous system in *Nephtys minuta* (Polychaeta). Zool. Zh. 58: 949-957.

Burke, R.D. 1978. Development of the larval digestive tract of echinoids. Ph.D. Thesis. University of Alberta.

Caine, E.A. 1980. Adaptations of a species of hermit crab (Decapoda, Paguridea) inhabiting sessile worm tubes. Crustaceana 38: 306-310.

Cameron, R.A. 1975. The initiation and early events of metamorphosis of sea urchins. Ph.D. Thesis. University of California, Santa Cruz.

Caullery, M. 1914. Sur les formes larvaires des annélides de la famille des Sabellariens (Hermelliens). Bull. Soc. zool. Fr. 39: 168-176.

Cazaux, C. 1964. Développement larvaire de Sabellaria alveolata Linné. Bull. Inst. Oceanogr. 62: 1-21.

Chia, F.S. 1972. Adaptive significance of marine invertebrates in assimilation of dissolved organic molecules from seawater. Chinese Bioscience 1: 41-48.

Chia, F.S. and R.D. Burke. 1978. Echinoderm metamorphosis: fate of larval structures. In: Settlement and Metamorphosis of Marine Invertebrate Larvae. Chia, F.S. and M.E. Rice (eds.). Elsevier/ North-Holland. New York.

Child, C.M. 1900. The early development of Arenicola and Sternapsis. Wilhelm Roux Arch. Entwicklungsmech. Organismen 9: 587-722.

Clark, R.B. 1978. Environmental determination of reproduction in polychaetes. In: Reproductive Ecology of Marine Invertebrates. Stancyk, S.E. (ed.). University of South Carolina Press. Columbia.

Cloney, R.A. and E. Florey. 1968. Ultrastructure of cephalopod chromatophore organs. Z. Zellforsch. mikrosk. Anat. 89: 250-280.

Crisp, D.J. 1967. Chemical factors inducing settlement in Crassostrea virginica Gmelin. J. Anim. Ecol. 36: 329-335.

Crisp, D.J. 1979. Dispersal and re-aggregation in sessile marine invertebrates, particularly barnacles. In: Biology and Systematics of Colonial Organisms. Larwood, G. and B.R. Rosen (eds.). Academic Press. New York.

Curtis, L.A. 1973. Aspects of the life cycle of Sabellaria vulgaris (Verrill) (Polychaeta:Sabellariidae) in Delaware Bay. Ph.D. Thesis. University of Delaware.

Curtis, L.A. 1978. Aspects of population dynamics of the polychaete Sabellaria vulgaris Verrill, in the Delaware Bay. Estuaries 1: 73-84.

Dales, R.P. 1952. The development and structure of the anterior region of the body in the Sabellariidae, with special reference to Phragmatopoma californica. Quart. J. micr. Sci. 93: 435-452.

Daly, J.M. 1963. Behavioural and secretory activity during tube construction by Platynereis dumerilli Aud. and M.Edw. (Polychaeta:Nereidae). J. mar. biol. Ass. U.K. 53: 521-529.

Day, R.L. and J.A. Blake. 1979. Reproduction and larval development of Polydora giardi Mensil (Polychaeta:

Spionidae). Biol. Bull. 156: 20-30.

Dorsett, D.A. 1976. The structure and function of proprioreceptors in soft-bodied invertebrates. In: Structure and Function of Proprioreceptors in the Invertebrates. Mill, P.J. (ed.). Chapman and Hall. London.

Dorsett, D.A. and R. Hyde. 1970. The epidermal glands of Nereis. Z. Zellforsch. mikrosk. Anat. 100: 219-230.

Drasche, R. von. 1885. Beiträge zur Entwicklung der Polychaeten. II. Entwicklung von Sabellaria spinulosa, Hermione hystrix, und einer Phyllodocide. Wien: Verlag von Carl Gerolds Sohn.

Dunlap, H.L. 1966. Oogenesis in Ctenophora. Ph.D. Thesis. University of Washington.

Eakin, R.M. and J.A. Westfall. 1964. Further observations on the fine structure of some invertebrate eyes. Z. Zellforsch. mikrosk. Anat. 62: 310-332.

Ebling, F.J. 1945. Formation and nature of the opercular chaetae of Sabellaria alveolata. Quart. J. micr. Sci. 85: 153-176.

Eckelbarger, K.J. 1975. Developmental studies of the post-settling stages of Sabellaria vulgaris (Polychaeta:Sabellariidae). Mar. Biol. 30: 137-149.

Eckelbarger, K.J. 1976. Larval development and population aspects of the reef-building polychaete Phragmatopoma lapidosa from the east coast of Florida. Bull. Mar. Sci. 26: 117-132.

Eckelbarger, K.J. 1977. Larval development of Sabellaria floridensis from Florida and Phragmatopoma californica from Southern California (Polychaeta:Sabellariidae), with a key to the sabellariid larvae of Florida and a review of development in the family. Bull. Mar. Sci. 27: 241-255.

Eckelbarger, K.J. 1978. Metamorphosis and settlement in the Sabellariidae. In: Settlement and Metamorphosis of Marine Invertebrate Larvae. Chia, F.S. and M.E. Rice (eds.). Elsevier/North-Holland. New York.

Eckelbarger, K.J. and F.S. Chia. 1976. Scanning electron microscopic observations on the larval development of the reef-building polychaete Phragmatopoma lapidosa. Can. J. Zool. 54: 2082-2088.

Eckelbarger, K.J. and F.S. Chia. 1978. Morphogenesis of larval cuticle in the polychaete Phragmatopoma lapidosa. Cell Tiss. Res. 186: 187-201.

Fauchald, K. 1974. Polychaete phylogeny: a problem in protostome evolution. Syst. Zool. 23: 493-506.

Fauchald, K. 1977. The polychaete worms. Definitions and keys to the orders, families, and genera. Natl. Hist. Mus. Los Angeles Co. Sci. Ser. 28.

Fauchald, K. and P.A. Jumars. 1979. The diet of worms: a study of polychaete feeding guilds. Oceanogr. Mar. Biol. Ann. Rev. 17: 193-284.

Franzén, Å. 1956. On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates. Zool. Bidr. Upps. 31: 355-482.

Goodrich, E.S. 1897. On the relation of the arthropod head to the annelid prostomium. Quart. J. micr. Sci. 40: 185-195.

Gustus, R.M. and R.A. Cloney. 1973. Ultrastructure of the larval compound setae of the polychaete *Nereis vexillosa* Grube. J. Morph. 140: 355-366.

Hannerz, L. 1956. Larval development of the polychaete families Spionidae Sars, Disomidae Mesnil and Poecilochaetidae n. fam. in the Gullmar Fjord (Sweden). Zool. Bidr. Upps. 31: 1-204.

Hanson, J. 1949. The histology of the blood system in oligochaetes and polychaetes. Biol. Rev. 24: 127-173.

Hartman, O. 1944. Polychaetous annelids. Part VI. Paraonidae, Magelonidae, Longosomidae, Ctenodrilidae, and Sabellariidae. Alan Hancock Pacif. Exped. 10: 311-389.

Hartman, O. 1969. Atlas of the Sedentariate Polychaetous Annelids from California. Alan Hancock Foundation. Los Angeles.

Hermans, C.O. 1966. The natural history and larval anatomy of *Armandia brevis* (Polychaeta: Opheliidae). Ph.D. Thesis. University of Washington.

Hermans, C.O. 1969. Fine structure of the segmental ocelli of *Armandia brevis* (Polychaeta: Opheliidae). Z. Zellforsch. mikrosk. Anat. 96: 361-371.

Hermans, C.O. 1978. Metamorphosis in the opheliid polychaete *Armandia brevis*. In: Settlement and Metamorphosis of Marine Invertebrate Larvae. Chia, F.S. and M.E. Rice (eds.). Elsevier/North Holland. New York.

Hermans, C.O. and R.A. Cloney. 1966. Fine structure of the prostomial eyes of *Armandia brevis* (Polychaeta: Opheliidae). Z. Zellforsch. mikrosk. Anat. 72: 583-596.

Holborow, P.L. 1971. The fine structure of the trochophore of *Harmothoë imbricata*. Proc. Eur. mar. Biol. Symp. 4: 237-246.

Holborow, P.L., M.S. Laverack, and V.C. Barber. 1969. Cilia and other surface structures of the trochophore of *Harmothoë imbricata* (Polychaeta). Z. Zellforsch. mikrosk. Anat. 98: 246-261.

Holborow, P.L. and M.S. Laverack, 1972. Presumptive photoreceptor structures of the trochophore of *Harmothoë imbricata* (Polychaeta). Mar. Behav. Physiol. 1: 139-156.

Horst, R. 1881. Sur la fécondation et la développement de l'*Hermella alveolata* M. Edw. Ann. Mag. Nat. Hist. 10: 75-76.

Humason, G.L. 1967. Animal Tissue Techniques. W.H. Freeman and Co. San Francisco.

Iwanoff, P. 1928. Die entwicklung de larvalsegments bei den anneliden. Z. Morph. Tiere 10: 62-161.

Klöckner, K. 1976. Zur ökologie von *Pomatoceros triqueter* (Serpulidae, Polychaeta). I. Reproduktionsablauf, substratwahl, wachstum und mortalität. Helgoländer wiss. Meeresunters. 28: 352-400.

Knight-Jones, E.W. 1953. Laboratory experiments on gregariousness during settling in Balanus balanoides and other barnacles. J. exp. Biol. 30: 584-598.

Korn, H. 1958. Vergleichend-embryologische untersuchungen an Harmothoë Kinberg 1857 (Polychaeta, Annelida), organogenese und neurosecretion. Z. Wiss. Zool. Abt. A. 16: 346-443.

Korn, H. 1960. Das larvale nervensystem von Pectinaria Lamarck und Nepthys Cuvier (Annelida, Polychaeta). Zool. Jahrb. 78: 427-456.

Kozloff, E.N. 1974. Keys to the Marine Invertebrates of Puget Sound, the San Juan Archipelago, and Adjacent Regions. University of Washington Press. Seattle.

Lacalli, T. Structure and development of the apical organ in trochophores of Spirabranchus polycerus, Phyllodoce maculata and Phyllodoce mucosa. In Press.

Lacalli, T. and J.R. Marsden. 1977. A reticulum of nerve-like cells from trochophores of Phyllodoce mucosa. Experientia 33: 952-954.

Lillie, R.S. 1905. The structure and development of the nephridia of Arenicola cristata Stimpson. Mitt. zool. Sta. Neap. 17; 341-405.

Luft, J.H. 1961. Improvement in epoxy resin embedding methods. J. Biochem. Biophys. Cytol. 9: 409-414.

Mackie, G.O., C.L. Singla and C. Thiriot-Quiévreux. 1976.

Nervous control of ciliary activity in gastropod larvae. Biol. Bull. 151: 182-199.

Manahan, D.T. 1980. Autoradiographic studies on the uptake of dissolved glycine by bivalve larvae. Am. Zool. 20: 869.

Marsden, J.R. and T. Lacalli. 1978. Morphology and behavior of the benthic larva of Arenicola cristata. Can. J. Zool. 56: 224-237.

Marsden, J.R. and L. Pawson. 1981. Metamorphosis of the larva of the polychaete Arenicola cristata. Morphology of the 'hour-glass' stage. Int. J. Invert. Reprod. 3: 133-143.

McNulty, J.K. and N.N. Lopez. 1969. Year-round production of ripe-gametes by benthic polychaetes in Biscayne Bay, Florida. Bull. Mar. Sci. 19: 945-954.

Michel, C. and E.J. DeVillez. 1978. Digestion. In: Physiology of Annelids. Mill, P.J. (ed.). Academic Press. New York.

Millonig, G. 1961. Advantages of a phosphate buffer for $OsO_4$ solutions in fixation. J. appl. Phys. 32: 1637-1646.

Moore, J.P. 1906. Additional new species of Polychaeta from the North Pacific. Proc. Acad. Nat. Sci. Pa. 58: 217-259.

Nott, J.A. 1973. Settlement of the larvae of Spirorbis spirorbis L. J. mar. biol. Assoc. U.K. 53: 437-453.

Novikoff, A.B. 1937. Sabellaria vulgaris. In: Culture Methods for Invertebrate Animals. Galtsoff et al. (eds.). Comstock. Ithaca, N.Y.

Novikoff, A.B. 1938. Embryonic determination in the annelid Sabellaria vulgaris. I. The differentiation of ectoderm and endoderm when separated by induced exogastrulation. Biol. Bull. 74: 198-210.

Novikoff, A.B. 1939. Surface changes in unfertilized and fertilized eggs of Sabellaria vulgaris. J. exp. Zool. 82: 217-237.

O'Clair, R.M. and R.A. Cloney. 1974. Patterns of morphogenesis mediated by dynamic microvilli: chaetogenesis in Nereis vexillosa. Cell Tiss. Res. 151: 141-158.

Orrhage, L. 1971. Light and electron microscope studies of some annelid setae. Acta zool. (Stockh.) 52: 157-169.

Orrhage, L. 1978. On the structure and evolution of the anterior end of the Sabellariidae (Polychaeta Sedentaria) with some remarks on the general organization of the polychaete brain. Zool. Jb. Anat. Bd. 100, S: 343-374.

Ospovat, M.F. 1978. Development of the nervous system in Phyllodoce ontogenesis: trochophore and metatrochophore. Zool. Zh. 57: 987-997.

Potswald, H.E. 1965. Reproductive biology and development of Spirorbis (Polychaeta, Serpulidae). Ph.D.

Thesis. University of Washington.

Potswald, H.E. 1971. A fine structural analysis of the epidermis and cuticle of the oligochaete Aeolosoma bengalense Stephenson. J. Morph. 135: 185-212.

Potswald, H.E. 1978. Metamorphosis in Spirorbis (Polychaeta). In: Settlement and Metamorphosis of Marine Invertebrate Larvae. Chia, F.S. and M.E. Rice (eds.). Elsevier/North-Holland. New York.

Quiévreux, C. 1962. Morphologie et anatomie des larves de Spirorbis vitreus (Fabricius) et Spirorbis malardi (Caullery et Mesnil) (Ann. Polychètes). Cahiers Biol. Marine 3:1-12.

Rees, C.P. 1976. Sand grain size distribution in tubes of Sabellaria vulgaris Verrill. Chesapeake Science 17: 59-61.

Reish, D.J. and G.C. Stephens. 1969. Uptake of organic material by aquatic invertebrates. V. The influence of age on uptake of glycine-$C^{14}$ by the polychaete Neanthes arenaceodentata. Mar. Biol. 3: 352-355.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. 17: 208-212.

Rice, S.A. and J.L. Simon. 1980. Intraspecific variation in the pollution indicator polychaete Polydora ligni (Spionidae). Ophelia 19: 79-115.

Richards, K.S. 1978. Epidermis and cuticle. In: Physiology of Annelids. Mill, P.J. (ed.).

Academic Press. New York.

Richardson, K.C., L. Jarrett, and E.H. Finke. 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. Stain Technol. 35: 313-323.

Rieger, R.M. and G.E. Rieger. 1976. Fine structure of the archiannelid cuticle and remarks on the evolution of the cuticle within the Spiralia. Acta zool. (Stockh.) 57: 53-68.

Rudall, K.M. 1963. The chitin/protein complexes of insect cuticles. In: Advances in Insect Physiology, Vol. I. Beament, J.W.L., J.E. Treherne, and V.B. Wigglesworth (eds.). Academic Press. New York.

Sarvala, J. 1971. Ecology of Harmothoë sarsi (Malmgren) (Polychaeta, Polynoidae) in the northern Baltic area. Ann. Zool. Fenn. 8: 231-309.

Schacter, S., E.R. Kandel, and R. Wooley. 1979a. Development of neurons in the abdominal ganglion of Aplysia californica. I. Axosomatic synaptic contacts. Dev. Biol. 71: 163-175.

Schacter, S., E.R. Kandel, and R. Wooley. 1979b. Development of neurons in the abdominal ganglion of Aplysia californica. II. Neuronal support cells. Dev. Biol. 71: 176-190.

Scheltema, R. 1971. The dispersal of the larvae of shoal-water benthic invertebrate species over long distances by ocean currents. Proc. Eur.

mar. Biol. Symp. 4: 7-28.

Schroeder, P.C. and C.O. Hermans. 1975. Annelida: Polychaeta. In: Reproduction of Marine Invertebrates Volume III: Annelids and Echiurans. Giese, A.C. and J.S. Pearse. (eds.). Academic Press. New York.

Segrove, F. 1941. The development of the serpulid *Pomatoceros triqueter* L. Quart. J. micr. Sci. 82: 467-540.

Sentz-Braconnet, E. 1968. Données ecologiques et biolgiques sur la fixation des Serpulidae dans le rade de Villefranche-sur Mer (Alpes Maritimes). Vie et Milieu 19 (B, océanogr.) 1: 109-132.

Shearer, C. 1911. On the development and structure of the trochophore of *Hydroides uncinatus* (*Eupomatus*). Quart. J. micr. Sci. 56: 543-591.

Siebers, D. and H.P. Bulhein. 1977. Salinity dependence, uptake kinetics, and specificity of amino acid absorption across the body surface of the oligochaete annelid *Enchytraeus albidus*. Helgoläender wiss. Meeresunters. 29: 473-592.

Smith, R.I. and J.T. Carlton. 1975. Light's Manual: Intertidal Invertebrates of the Central California Coast. University of California Press. Berkeley.

Storch, V. and U. Welsch. 1972. The ultrastructure of the epidermal mucous cells in marine invertebrates (Nemertini, Polychaeta, Prosobranchia, Opisthobranchia).

Mar. Biol. 13: 167-175.

Strathmann, M. 1974. Methods in Development Series. Friday Harbor Laboratories, Friday Harbor, Wash., USA.

Strathmann, R.R., T.L. Jahn, and J.R.C. Fonseca. 1972. Suspension feeding by marine invertebrate larvae: clearance of particles by ciliated bands of a rotifer, pluteus, and trochophore. Biol. Bull. 142: 508-519.

Straughan, D. 1972. Ecological study of Mercierella enigmatica Fauvel (Annelida: Polychaeta) in the Brisbane river. J. Anim. Ecol. 41: 93-136.

Tempel, D. and W. Westheide. 1980. Uptake and incorporation of dissolved amino acids by interstitial Turbellaria and Polychaeta and their dependence on temperature and salinity. Mar. Ecol. Prog. Ser. 3: 41-50.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25: 1-45.

Treadwell, A.L. 1901. The cytogeny of Podarke obscura. J. Morph. 17: 399-486.

Waterman, A.J. 1934. Observations on reproduction, pre-maturation and fertilization in Sabellaria vulgaris. Biol. Bull. 67: 97-114.

Watson, M.L. 1958. Staining of tissue for electron microscopy with heavy metals. II. Application of solutions containing lead and barium. J. Biophys. Biochem. Cytol. 4: 475-479.

Werner, B. 1953. Beobachtungen über den Nahrungserwerb und die metamorphose der metatrochophora von *Chaetopterus variopedatus* Reiner and Claparède. Heloglaender Wiss. Meeresunters. 5: 93-102.

Westheide, W. and R.M. Rieger. 1978. Cuticle ultrastructure of hesionid polychaetes (Annelida). Zoomorphologie 91: 1-18.

Williams, J.B. 1978. Studies of the epidermis of *Temnocyphala* III. Scanning electron microscope study of the epidermal surface of *Temnocyphala dendyi*. Aust. J. Zool. 26: 217-221.

Wilson, D.P. 1929. The larvae of the British sabellarians. J. mar. biol. Assoc. U.K. 15: 221-269.

Wilson, D.P. 1932. On the mitraria larva of *Owenia fusiformis* delle Chiaje. Phil. Trans. Roy. Soc. London B221: 231-334.

Wilson, D.P. 1936a. The development of the sabellid *Branchiomma vesiculosum*. Quart. J. micr. Sci. 78: 543-603.

Wilson, D.P. 1936b. Notes on the early stages of two polychaetes, *Nepthys hombergi* Lamarck and *Pectinaria koreni* Malmgren. J. mar. biol. Assoc. U.K. 21: 305-310.

Wilson, D.P. 1958. Some problems in larval ecology related to the localized distribution of bottom animals. In: Perspectives in Marine Biology. Buzzati-Traverso, A.A. (ed.). University of

California Press. Berkeley.

Wilson, D.P. 1968a. Some aspects of the development of eggs and larvae of *Sabellaria alveolata* (L.). J. mar. biol. Assoc. U.K. 48: 367-386.

Wilson, D.P. 1968b. The settlement behavior of the larvae of *Sabellaria alveolata* (L.). J. mar. biol. Assoc. U.K. 48: 387-435.

Wilson, D.P. 1970a. Additional observations on larval growth and settlement in *Sabellaria alveolata*. J. mar. biol. Assoc. U.K. 50: 1-31.

Wilson, D.P. 1970b. The larvae of *Sabellaria spinulosa* and their settlement behavior. J. mar. biol. Assoc. U.K. 50: 33-52.

Wilson, D.P. 1977. The distribution, development and settlement of the sabellarian polychaete *Lygdamis muratus* (Allen) near Plymouth. J. mar. biol. Assoc. U.K. 57: 761-792.

Wilson, E.B. 1892. The cell lineage of *Nereis*. A contribution to the cytogeny of the annelid body. J. Morph. 6: 361-466.

Winesdorfer, J.E. 1967. Marine annelids: *Sabellaria*. In: Methods in Developmental Biology. Wilt, F.M. and N.K. Wessells (eds.). Thomas Y. Crowell Company. New York.

Woltreck, R. 1905. Zur kopffrage der anneliden. Neue beobachtungen zur metamorphose der hatschekschen *Polygordius* larve, insbesondre über die genese der hohlräume, nephriden und blutgefässe der kopfregion. Verh. Zool. Gesells. Leipzig: 154-186.

Wu, B. and S. Ruiping. 1979. On the occurrence of two sabellariid worms in the Yellow Sea, with notes on their larval development. Acta Zool. Sinica. 25: 130-142.